Exhibit 158, part 3

American Journal of Epidemiology Vol. 170, No. 5
DOI: 10.1093/aje/kwp176
Advance Access publication July 15, 2009

Original Contribution

Ovarian Cancer Risk Factors in African-American and White Women

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Initially submitted February 5, 2009; accepted for publication May 27, 2009.

Ovarian cancer is the most lethal gynecologic malignancy in both African-American and white women. Although prevalences of many ovarian cancer risk factors differ markedly between African Americans and whites, there has been little research on how the relative contributions of risk factors may vary between racial/ethnic groups. Using data from a North Carolina case-control study (1999–2008), the authors conducted unconditional logistic regression analyses to calculate odds ratios and 95% confidence intervals for ovarian cancer risk factors in African-American (143 cases, 189 controls) and white (943 cases, 868 controls) women and to test for interactions by race/ethnicity. They also calculated attributable fractions within each racial/ethnic group for the modifiable factors of pregnancy, oral contraceptive use, tubal ligation, and body mass index. Many risk factors showed similar relations across racial/ethnic groups, but tubal ligation and family history of breast or ovarian cancer showed stronger associations among African Americans. Younger age at menarche was associated with risk only in white women. Attributable fractions associated with tubal ligation, oral contraceptive use, and obesity were markedly higher for African Americans. The relative importance of ovarian cancer risk factors may differ for African-American women, but conclusions were limited by the small sample. There is a clear need for further research on etiologic factors for ovarian cancer in African-American women.

African Americans; case-control studies; ovarian neoplasms

Abbreviations: BMI, body mass index; CI, confidence interval.

Ovarian cancer is the eighth most common cancer among both white and African-American women and the fifth most common cause of cancer death in the United States (1, 2). African-American women have lower incidence rates than white women (10.1 cases/100,000 women vs. 14.1 cases/100,000 women) but poorer 5-year survival (1). Despite the importance of ovarian cancer as a major cause of morbidity and mortality, there has been very little research on ovarian cancer among African Americans. Only 2 published papers have focused on risk factors for ovarian cancer among African Americans: 1 on a case-control study with 84 cases (3) and 1 on a multicenter analysis of 7 case-control studies involving 110 cases (4). Both of these reports had findings that were consistent with the major reproductive risk factors identified in white women, including inverse associations with parity and oral contraceptive use (3, 4), but some racial/ethnic differences were noted, including the absence of a protective effect for breastfeeding and no increased risk associated with a family history of ovarian cancer among African Americans (3).

As has been reported by John et al. (4), Ness et al. (3), and other authors (5–12), the prevalence of many factors associated with risk of ovarian cancer varies markedly between African Americans and whites. African-American women tend to have a greater number of pregnancies (5, 7), a higher prevalence of tubal ligation (6), a lower prevalence of endometriosis (9), and less use of menopausal hormones (5, 10), all of which would be associated with a lower incidence of ovarian cancer. They also tend to have an earlier age at menarche (11), are more likely to be obese (12), and are less likely to breastfeed (8), which could contribute to higher risk of ovarian cancer. Because most epidemiologic

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studies of ovarian cancer have enrolled very few African-American women, there is little information on the relative importance of these risk factors among African-American women as compared with white women and the extent to which differences in the prevalence of established risk factors can explain the lower incidence of ovarian cancer among African Americans.

In this paper, we use data from the North Carolina Ovarian Cancer Study to compare risk factors for ovarian cancer among African-American and white women. We also calculate population attributable fractions for risk factors that are both modifiable and show considerable racial/ethnic differences in prevalence to evaluate the relative proportions of cases in African-American and white women that are associated with these factors.

MATERIALS AND METHODS

The North Carolina Ovarian Cancer Study was a population-based, case-control study of epithelial ovarian cancer that was conducted in a 48-county region of North Carolina between 1999 and 2008. Newly diagnosed cases of epithelial ovarian cancer were identified through the North Carolina Central Cancer Registry using a rapid case ascertainment system. Pathology reports for eligible cases were sent to the study office at Duke University Medical Center, and consent to contact the women was requested from the treating physicians. Eligible cases were aged 20–74 years at diagnosis, had no prior history of ovarian cancer, resided in the study area, and were cognitively able to give consent and to complete an interview in English. All cases underwent standardized histopathologic review by the study pathologist for confirmation of the diagnosis. Control women were frequency-matched by age and race/ethnicity to the cases and were recruited from the same geographic region using list-assisted random digit dialing. The eligibility criteria were the same as those for the cases; in addition, the controls could not have had a bilateral oophorectomy.

The response rate among the cases was 66.5%, with nonparticipation being due to death (4.0%), debilitating illness (2.6%), physician refusal (4.7%), patient refusal (11.5%), or an inability to locate the patient (10.7%). Among potential controls, screening for eligibility could not be completed for 14% of phone numbers. Seventy-three percent of potential controls who passed eligibility screening agreed to be sent information about the study, and 60.1% of those consented to be in the study. Nonparticipation was due to refusal (27.4%) or an inability to contact the person (8.8%). Response rates were lower for African Americans than for whites (56.6% and 68.3%, respectively, for cases and 49.7% and 63.7%, respectively, for controls).

A total of 1,114 cases were enrolled, of whom 943 (84.6%) were white, 143 (12.8%) were African-American, and 28 (2.5%) were of other races/ethnicities. Among the 1,086 controls, 868 (79.9%) were white, 189 (17.4%) were African-American, and 29 (2.7%) were of other races/ethnicities. The analyses in this report were limited to women whose self-reported race/ethnicity was either white or African-American. The study protocol was approved by the Duke University Medical Center Institutional Review Board and by the human subjects committees at the North Carolina Central Cancer Registry and each hospital where cases were identified.

Table 1. Clinical and Histologic Characteristics of Epithelial Ovarian Cancer Cases in African-American and White Women, North Carolina Ovarian Cancer Study, 1999–2008

	Whites		African Americans		*P* Value[a]
	No.	%	No.	%	
All cases	(n = 943)		(n = 143)		
Invasive tumor	746	79.4	111	77.6	0.64
Low-malignant-potential tumor	194	20.6	32	22.4	
Missing data	3		0		
Invasive cases only	(n = 746)		(n = 111)		
Histologic type					
Serous	419	56.2	67	60.4	0.05
Clear-cell	82	11.0	2	1.8	
Endometrioid	116	15.5	19	17.1	
Mucinous	39	5.2	6	5.4	
Other	90	12.1	17	15.3	
Stage					
I or II	245	33.1	25	22.7	0.04
III or IV	496	66.9	85	77.3	
Missing data	5		1		
Grade					
Well-differentiated	93	12.9	18	16.8	0.12
Moderately differentiated	197	27.2	36	33.6	
Poorly differentiated or undifferentiated	433	59.9	53	49.5	
Missing data	23		4		

[a] *P* values were derived from chi-squared analyses.

Nurse-interviewers conducted in-person visits at which they obtained written informed consent, administered a 90-minute questionnaire, drew a blood sample, and performed anthropometric measurements (height, weight, and waist and hip circumferences). Information obtained with the questionnaire included family history of cancer; menstrual characteristics such as age at menarche and cycle length; reproductive history, including age at each pregnancy, pregnancy duration and outcome, and duration of breastfeeding; type, timing, and duration of hormone and contraceptive use; and lifestyle characteristics such as smoking history, alcohol consumption during the 5 years before interview, and physical activity. A life-events calendar, which marked milestones such as marriages and births, was used to aid recall of reproductive history and hormone use. Pictures of oral contraceptives, menopausal hormones, and certain other medications were also used to assist with recall.

Statistical analysis

Chi-squared analyses were used to compare clinical and histologic characteristics of cases between African Americans

Table 2. Characteristics of Invasive Epithelial Ovarian Cancer Cases and Controls, by Race/Ethnicity, North Carolina Ovarian Cancer Study, 1999–2008

	Whites						African Americans					
	Cases (*n* = 746)		Controls (*n* = 868)		OR[a]	95% CI	Cases (*n* = 111)		Controls (*n* = 189)		OR[a]	95% CI
	No.	%	No.	%			No.	%	No.	%		
Age, years												
20–39	38	5.1	81	9.3			11	9.9	22	11.6		
40–49	136	18.2	170	19.6			23	20.7	46	24.3		
50–59	239	32.0	261	30.1			37	33.3	67	35.4		
60–69	232	31.1	240	27.6			31	27.9	40	21.2		
70–74	101	13.5	116	13.4			9	8.1	14	7.4		
Age at menarche, years												
<12	181	24.4	157	18.2	1.00	Referent	28	25.5	53	28.0	1.00	Referent
≥12	562	75.6	708	81.8	0.67	0.53, 0.86	82	74.5	136	72.0	1.08	0.63, 1.85
Missing data	3		3				1		0			
No. of pregnancies								11				
0	120	16.1	87	10.0	1.00	Referent	14	12.6	11	5.8	1.00	Referent
1–2	319	42.8	348	40.1	0.62	0.45, 0.85	31	27.9	71	37.6	0.34	0.14, 0.82
≥3	307	41.2	433	49.9	0.45	0.33, 0.62	66	59.5	107	56.6	0.44	0.19, 1.05
P-trend						<0.0001	0		0			0.25
Age at first pregnancy, years												
<20	173	27.6	202	25.9	1.00	Referent	56	58.3	94	52.8	1.00	Referent
20–24	276	44.1	333	42.7	0.93	0.72, 1.21	30	31.3	52	29.2	0.98	0.56, 1.72
25–29	137	21.9	151	19.4	1.09	0.80, 1.49	8	8.3	19	10.7	0.73	0.30, 1.79
30–34	31	5.0	79	10.1	0.50	0.31, 0.79	1	1.0	10	5.6	0.18	0.02, 1.45
≥35	9	1.4	14	1.8	0.77	0.33, 1.84	1	1.0	3	1.7	0.65	0.07, 6.45
Missing data	120		89				15		11			
P-trend						0.0004						0.15
Age at last pregnancy, years												
<20	19	3.0	18	2.3	1.00	Referent	11	11.7	13	7.3	1.00	Referent
20–24	134	21.4	147	18.9	0.79	0.39, 1.57	24	25.5	35	19.8	0.82	0.31, 2.14
25–29	233	37.3	258	33.1	0.76	0.38, 1.49	25	26.6	51	28.8	0.57	0.22, 1.45
30–34	161	25.8	230	29.5	0.60	0.30, 1.18	22	23.4	48	27.1	0.54	0.21, 1.40
≥35	78	12.5	126	16.2	0.53	0.26, 1.08	12	12.8	30	16.9	0.43	0.15, 1.23
Missing data	121		89				17		12			
P-trend						<0.0001						0.04
Ever breastfeeding												
No	521	69.8	542	62.4	1.00	Referent	75	67.6	135	71.4	1.00	Referent
Yes	225	30.2	326	37.6	0.73	0.59, 0.90	36	32.4	54	28.6	1.16	0.69, 1.93
Missing data	0		0				0		0			
Tubal ligation												
No	559	75.0	579	66.8	1.00	Referent	77	69.4	93	49.2	1.00	Referent
Yes	186	25.0	288	33.2	0.68	0.54, 0.84	34	30.6	96	50.8	0.43	0.26, 0.71
Missing data	1		1				0		0			
Duration of oral contraceptive use, years												
Never use	244	34.5	239	28.3	1.00	Referent	47	43.9	58	32.2	1.00	Referent
<1	99	14.0	92	10.9	1.09	0.77, 1.52	15	14.0	14	7.8	1.36	0.59, 3.14
1–<5	166	23.4	228	27.0	0.75	0.57, 0.99	24	22.4	57	31.7	0.54	0.28, 1.04
≥5	199	28.1	285	33.8	0.73	0.55, 0.96	21	19.6	51	28.3	0.53	0.27, 1.03
Missing data	38		24				4		9			

Table continues

Table 2. Continued

	Whites						African Americans					
	Cases (*n* = 746)		Controls (*n* = 868)		OR[a]	95% CI	Cases (*n* = 111)		Controls (*n* = 189)		OR[a]	95% CI
	No.	%	No.	%			No.	%	No.	%		
Use of menopausal hormones												
No	276	37.0	456	52.6	1.00	Referent	75	68.8	148	78.3	1.00	Referent
Yes	470	63.0	411	47.4	1.85	1.50, 2.28	34	31.2	41	21.7	1.54	0.90, 2.66
Missing data	0		1				2		0			
Hysterectomy												
No	537	72.2	667	76.9	1.00	Referent	82	73.9	145	76.7	1.00	Referent
Yes	207	27.8	200	23.1	1.22	0.97, 1.54	29	26.1	44	23.3	1.07	0.61, 1.87
Missing data	2		1				0		0			
History of infertility												
No	651	87.3	783	90.2	1.00	Referent	102	91.9	175	92.6	1.00	Referent
Yes	95	12.7	85	9.8	1.38	1.01, 1.89	9	8.1	14	7.4	1.13	0.47, 2.73
Missing data	0		0				0		0			
History of endometriosis												
No	650	87.7	793	92.3	1.00	Referent	109	98.2	184	98.4	1.00	Referent
Yes	91	12.3	66	7.7	1.76	1.26, 2.46	2	1.8	3	1.6	1.16	0.19, 7.08
Missing data	5		9				0		2			
First-degree family history of breast or ovarian cancer												
No	582	78.1	720	83.1	1.00	Referent	69	62.2	159	84.1	1.00	Referent
Yes	163	21.9	146	16.9	1.33	1.04, 1.71	42	37.8	30	15.9	3.15	1.82, 5.45
Missing data	1		2				0		0			
Talc use												
No	328	59.6	325	61.0	1.00	Referent	45	54.2	75	56.0	1.00	Referent
Yes	222	40.4	208	39.0	1.04	0.82, 1.33	38	45.8	59	44.0	1.19	0.68, 2.09
Missing data	196		335				28		55			
Body mass index[b] 1 year before diagnosis or interview												
<25	312	43.3	369	43.7	1.00	Referent	17	15.9	31	17.1	1.00	Referent
25–<30	212	29.4	256	30.3	0.96	0.76, 1.22	26	24.3	58	32.0	0.84	0.39, 1.78
30–<35	114	15.8	124	14.7	1.08	0.80, 1.45	22	20.6	43	23.8	0.94	0.43, 2.07
≥35	83	11.5	95	11.3	1.04	0.75, 1.45	42	39.3	49	27.1	1.62	0.79, 3.35
Missing data	25		24				4		8			
Height, m												
<1.6	195	26.2	242	27.9	1.00	Referent	25	22.7	57	30.2	1.00	Referent
1.6–<1.7	430	57.8	483	55.8	1.13	0.90, 1.42	64	58.2	102	54.0	1.48	0.84, 2.62
≥1.7	119	16.0	141	16.3	1.11	0.81, 1.51	21	19.1	30	15.9	1.74	0.83, 3.65
Missing data	2		2				1		0			

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Adjusted for age.
[b] Weight (kg)/height (m)^2.

and whites. Unconditional logistic regression analyses were used to calculate age-adjusted and multivariable-adjusted odds ratios and 95% confidence intervals separately for each racial/ethnic group. Variables included in the race/ethnicity-specific multivariable models were age, age at menarche, number of pregnancies, duration of oral contraceptive use, history of tubal ligation, family history of breast and ovarian cancer, and body mass index (BMI; weight (kg)/height (m)^2). The variables included in multivariable models were selected a priori and included the most well-established risk

factors for ovarian cancer as well as BMI, because of the pronounced racial/ethnic differences in the prevalence of obesity. We also conducted multivariable analyses limited to parous women that included all of the above variables plus breastfeeding. Finally, to test for interactions, we fitted models for women of both racial/ethnic groups combined which included a term for race/ethnicity and product terms for race/ethnicity $\times$ age at menarche, race/ethnicity $\times$ breastfeeding, and race/ethnicity $\times$ family history of breast or ovarian cancer.

Population attributable fractions were calculated using the method described by Bruzzi et al. (13) for the potentially modifiable factors tubal ligation (yes vs. no), oral contraceptive use ($\geq$1 year vs. <1 year), history of pregnancy (ever vs. never), and BMI (<30 vs. $\geq$30). For these analyses, the reference categories were assigned to the lower risk category (i.e., having had a tubal ligation, oral contraceptive use for $\geq$1 year, ever being pregnant, and BMI <30) so the attributable fraction could be interpreted as the proportion of cases that theoretically could be eliminated if all women in the population were shifted to the low risk category.

RESULTS

The tumor characteristics of the ovarian cancer cases are presented in Table 1 by race/ethnicity. The proportions of cases that were invasive were similar for African Americans and whites (78% and 79%, respectively). Because low-malignant-potential ovarian cancer may be etiologically distinct from invasive cancer (14, 15), we focused the remainder of our analyses on invasive disease. Among invasive cases, the most important histologic differences were that tumors in African Americans were less likely to be clear-cell and more likely to be of a histologic type other than the 4 primary types (serous, endometrioid, mucinous, and clear-cell). African-American women were more likely to be diagnosed with higher-stage disease and somewhat less likely to have poorly differentiated tumors, although the differences in grade were not statistically significant.

Comparisons of risk factors for ovarian cancer among African-American and white women are presented in Table 2. Because age-matching was based on all cases but this analysis was restricted to invasive cases, who are on average older than low-malignant-potential cases, the age distribution of the controls was slightly younger than that of the cases.

In age-adjusted analyses, many of the major reproductive factors that have been associated with ovarian cancer risk among white women were similarly related to risk among African-American women. Women who were parous, had a later age at last pregnancy, had used oral contraceptives for 1 year or more, or had had a tubal ligation were at reduced risk of invasive ovarian cancer; however, there was not strong evidence of a linear relation with number of pregnancies for African-American women. History of infertility or endometriosis was associated with a significantly increased risk for white women and a modestly but not significantly increased risk for African-American women. Family history of breast or ovarian cancer in a first-degree relative was associated with increased risk in both racial/ethnic groups, with a stronger association among African Americans. Later age at menarche and history of ever breastfeeding were associated with reduced risk in white women, whereas no association was observed among African Americans. Analyses of anthropometric characteristics suggested that taller height and BMI $\geq$35 may be associated with risk among African-American women but not among white women.

In multivariable models (Table 3), results were generally similar to those observed in the age-adjusted models. The association with age at menarche $\geq$12 years appeared to differ by race/ethnicity, with an odds ratio of 1.30 (95% confidence interval (CI): 0.67, 2.53) for African Americans rather than the expected inverse association. The strength of the association with family history of breast or ovarian cancer also appeared to differ by race/ethnicity. *P* values for the interaction terms were 0.032 for race/ethnicity $\times$ family history and 0.068 for race/ethnicity $\times$ age at menarche. In models limited to parous women that included all of the variables in Table 3 plus history of breastfeeding, white women who had breastfed had a nonsignificantly reduced risk (odds ratio = 0.83, 95% CI: 0.65, 1.06), whereas there was no suggestion of a protective effect among African-American women (odds ratio = 1.09, 95% CI: 0.57, 2.07).

In addition to some differences between African Americans and whites in the magnitude of associations with certain risk factors, there were marked racial/ethnic differences in the prevalences of a number of risk factors considered. For example, prevalences in African-American and white controls, respectively, were 29% and 18% for age at menarche less than 12 years, 6% and 10% for nulligravidity, 51% and 33% for tubal ligation, and 51% and 26% for BMI $\geq$30 (Table 3). We therefore hypothesized that the relative contribution of established risk factors for ovarian cancer could vary considerably between African Americans and whites. To address this, we calculated population attributable fractions for the potentially modifiable risk factors of pregnancy, oral contraceptive use, BMI, and tubal ligation. As Table 4 shows, the attributable fractions for not having a tubal ligation, high BMI, and no oral contraceptive use were considerably higher for African Americans than for whites, reflecting the stronger associations and/or higher prevalence of these factors among African Americans.

DISCUSSION

Our analyses of ovarian cancer risk factors in African-American and white women show similar relations for several characteristics, including inverse associations with parity, oral contraceptive use, and tubal ligation, but there are also suggestions of racial/ethnic differences in either the direction or the magnitude of association for other risk factors. History of breastfeeding and later age at menarche were both associated with reduced risk among whites, whereas these associations were absent among African Americans. Family history of breast or ovarian cancer was associated with increased risk for both African Americans and whites, but the association was considerably stronger for African-American women. We considered the possibility that the

Table 3. Odds Ratios for Invasive Epithelial Ovarian Cancer (Multivariable Logistic Regression Models) in African-American and White Women, North Carolina Ovarian Cancer Study, 1999–2008

	Whites						African Americans					
	Cases		Controls		OR[a]	95% CI	Cases		Controls		OR[a]	95% CI
	No.	%	No.	%			No.	%	No.	%		
Age, years (continuous variable)	715		837		1.01	1.00, 1.02	106		181		1.00	1.00, 1.02
No. of pregnancies												
0	114	15.9	84	10.0	1.00	Referent	14	13.2	11	6.1	1.00	Referent
1–2	306	42.8	332	39.7	0.66	0.47, 0.94	29	27.4	68	37.6	0.28	0.09, 0.86
≥3	295	41.3	421	50.3	0.46	0.32, 0.65	63	59.4	102	56.4	0.52	0.17, 1.62
Age at menarche, years												
<12	172	24.1	151	18.0	1.00	Referent	26	24.5	52	28.7	1.00	Referent
≥12	543	75.9	686	82.0	0.65	0.50, 0.84	80	75.5	129	71.3	1.30	0.67, 2.53
Tubal ligation												
No	535	74.8	561	67.0	1.00	Referent	73	68.9	89	49.2	1.00	Referent
Yes	180	25.2	276	33.0	0.74	0.58, 0.94	33	31.1	92	50.8	0.43	0.24, 0.80
Duration of oral contraceptive use, years												
Never use	233	34.1	225	27.6	1.00	Referent	45	43.3	55	32.0	1.00	Referent
<1	95	13.9	88	10.8	1.18	0.82, 1.69	15	14.4	14	8.1	1.89	0.73, 4.95
1–<5	162	23.7	222	27.2	0.78	0.58, 1.05	23	22.1	55	32.0	0.72	0.34, 1.53
≥5	193	28.3	281	34.4	0.73	0.54, 0.97	21	20.2	48	27.9	0.52	0.24, 1.15
Missing data	32		21				2		9			
Family history of breast or ovarian cancer												
No	559	78.2	697	83.3	1.00	Referent	66	62.3	153	84.5	1.00	Referent
Yes	156	21.8	140	16.7	1.31	1.00, 1.72	40	37.7	28	15.5	2.73	1.45, 5.14
Body mass index[b] 1 year before diagnosis/interview												
<25	309	43.2	368	44.0	1.00	Referent	17	16.0	31	17.1	1.00	Referent
25–<30	211	29.5	254	30.3	0.92	0.71, 1.18	26	24.5	58	32.0	0.96	0.40, 2.30
30–<35	112	15.7	122	14.6	1.17	0.85, 1.61	22	20.8	43	23.8	1.32	0.53, 3.26
≥35	83	11.6	93	11.1	1.03	0.72, 1.47	41	38.7	49	27.1	1.52	0.65, 3.56

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Adjusted for all of the variables in the table.
[b] Weight (kg)/height (m)^2.

stronger association in African-American women was due to inaccurate reporting; however, the prevalences of a family history of breast or ovarian cancer were very similar among African-American and white controls, which argues against there being differential reporting of family history across racial/ethnic groups.

Although these observed racial/ethnic differences in the magnitude or direction of associations with established ovarian cancer risk factors are intriguing, the limitations of our analyses must be acknowledged. The North Carolina Ovarian Cancer Study included more African-American women than any other study of ovarian cancer, but the relatively small sample made it difficult to ascertain which associations were true associations and which were chance findings.

The modest sample size also precluded us from conducting analyses within subgroups defined by either menopausal status or histologic type. Several reports have suggested that reproductive risk factors and high BMI are more strongly associated with premenopausal disease (16–23). However, with only 38 premenopausal African-American cases in our study population, analyses stratified by menopausal status would not have yielded meaningful results. Similarly, the sample was too small for us to explore differences in risk factors by histologic subtype. The relatively small number of African-American cases also led us to dichotomize some

Table 4. Odds Ratios for Invasive Epithelial Ovarian Cancer and Population Attributable Fractions for Selected Ovarian Cancer Risk Factors in African-American and White Women, North Carolina Ovarian Cancer Study, 1999–2008

	Whites					African Americans				
	No. of Cases	No. of Controls	OR[a]	95% CI	AF	No. of Cases	No. of Controls	OR[a]	95% CI	AF
Tubal ligation										
Yes	168	269	1.00	Referent	0.204	33	91	1.00	Referent	0.341
No	515	547	1.37	1.08, 1.73		71	81	2.00	1.15, 3.48	
Body mass index[b]										
<30	494	611	1.00	Referent	0.030	42	86	1.00	Referent	0.209
≥30	183	205	1.12	0.89, 1.42		62	86	1.54	0.91, 2.62	
Duration of oral contraceptive use, years										
≥1	355	503	1.00	Referent	0.119	44	103	1.00	Referent	0.245
<1	328	313	1.33	1.06, 1.67		60	69	1.74	0.99, 3.05	
Ever being pregnant										
Yes	575	734	1.00	Referent	0.052	91	164	1.00	Referent	0.079
No	108	82	1.49	1.06 2.08		14	8	2.43	0.88, 6.73	

Abbreviations: AF, attributable fraction; CI, confidence interval; OR, odds ratio.
[a] Adjusted for all of the variables in the table, as well as age, age at menarche, family history of breast or ovarian cancer, and breastfeeding.
[b] Weight (kg)/height (m)^2.

variables of interest in our analyses and dictated that we limit the number of potential confounders evaluated in our multivariable models. A larger sample would have afforded us the opportunity to further explore the effects of the timing and duration of oral contraceptive use and the timing of pregnancies or tubal ligation.

Additional limitations of our analysis included those related to the case-control method. The possibility of bias being introduced due to nonparticipation of ovarian cancer cases and controls should be considered. Although we used rapid case ascertainment to identify cases within 2 months of diagnosis and the median time to case interview was 4.5 months, which should have minimized survival bias, there is a possibility that cases who participated differed from those who did not. When we compared the tumor characteristics of ovarian cancer cases who were identified as eligible but did not participate (because of death, lack of physician consent, participant refusal, or inability to contact them) with the tumor characteristics of cases who did participate, we found that the proportion of invasive cases was slightly smaller among participants than among nonparticipants. This is not a surprising finding, given that cases who died quickly or for whom physicians did not give consent were more likely to have advanced disease. Among the invasive cases that were the focus of most of our analyses, we found no statistically significant differences in the proportions of higher-stage cancers between participants and nonparticipants. The racial/ethnic differences in histologic type that we observed among participants (i.e., a lower proportion of clear-cell tumors and a higher proportion of tumors of "other" histologic types among African Americans) were also observed among the nonparticipating cases. Thus, the invasive cases enrolled in the study appeared to be representative of the ovarian cancer cases diagnosed in our catchment area.

Nonparticipation also has the potential to introduce bias if participating cases and controls differ from persons who decline to participate in the study. Although we had no risk factor information on nonparticipants with which to assess their similarity with women who participated in the study, the associations we observed for white women within our study population are consistent with established ovarian cancer risk factors, which argues against our results' being biased due to nonparticipation.

Despite the limited sample of African-American women, the descriptive characteristics of our population and the attributable fraction analyses suggest that the relative importance of ovarian cancer risk factors may vary between African Americans and whites because of the substantial differences in the prevalence and strength of associations with factors such as tubal ligation and obesity. Tubal ligation, which had a stronger association with ovarian cancer among African Americans and is considerably more common among African Americans in our study population as well as in national surveys (6), could be an important explanatory factor for the lower rates of ovarian cancer among African Americans.

Obesity, which has shown modest associations with ovarian cancer risk in the majority of studies (22, 24–26), may be a considerably more important risk factor for African-American women, as evidenced by the markedly higher attributable fraction for obesity that we observed in our data. Consistent with national statistics (12), our data showed a much higher prevalence of obesity among African Americans than among whites. In particular, severe obesity

(BMI ≥35), which had a threefold higher prevalence among African Americans than among whites in our study, may be especially relevant as a risk factor for ovarian cancer among African Americans. Some investigators have reported either that associations between BMI and ovarian cancer risk were present only for persons with very high BMIs or that the relations were considerably stronger for women in the highest BMI categories (27, 28). Other investigators have found that the association between obesity and ovarian cancer was present only among premenopausal women or was much stronger for premenopausal ovarian cancer than for postmenopausal ovarian cancer (21, 22). Because the markedly higher prevalence of obesity among African-American women is apparent even among adults aged 20–39 years (12), African-American women may be at higher risk for ovarian cancer diagnosed at a younger age. This is consistent with the higher proportion of premenopausal ovarian cancer cases in African Americans as compared with whites (34% vs. 26%) and the younger mean age at diagnosis (54.8 years vs. 57.4 years) that we observed in our population and that has been reported in Surveillance, Epidemiology, and End Results data (1). The younger age at diagnosis also may be related to the stronger association with family history of breast or ovarian cancer among African-American women, which could be indicative of higher genetic risk.

Our data suggest that the relative importance of ovarian cancer risk factors may vary between African-American and white women because of differences in the prevalence of and strength of associations with characteristics such as tubal ligation, pregnancy, and obesity. However, conclusions that can be drawn from our data are limited by the small number of African Americans in our analysis, despite our study population's having more African-American women than any other existing study of ovarian cancer. Because ovarian cancer is a leading cause of cancer mortality in African Americans, there is a clear need for additional studies in order to deepen our understanding of causative and protective factors in this population.

ACKNOWLEDGMENTS

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This work was supported by the National Institutes of Health (grant R01-CA76016 to J. M. S.) and the Department of Defense (grant DAMD17-02-1-0666 to A. B.).

Conflict of interest: none declared.

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OPEN

Epidemiology Publish Ahead of Print
DOI: 10.1097/EDE.0000000000000434

The association between talc use and ovarian cancer: a retrospective case-control study in two US states

Running Title: Ovarian cancer and talc

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Conflicts of Interest and Source of Funding:

Dr. Cramer reports being paid for expert testimony in litigation related to ovarian cancer. Ms. Vitonis reports being paid for programming work related to the same litigation. No other disclosures are reported. This work was supported by the National Institutes of Health (Grant numbers [R01CA054419, P50CA105009, R01CA67272,]), the Department of Defense Congressionally Directed Medical Research Programs (W81XWH-10-1-0280), and the Department of Obstetrics and Gynecology, Brigham and Women's Hospital.

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Abstract

Background: Multiple studies of ovarian cancer and genital talc use have led only to consensus about possible carcinogenicity. Seeking greater clarity, we examined this association in 2041 cases with epithelial ovarian cancer and 2100 age-and-residence-matched controls.

Methods: We defined genital talc use as regular application to the genital/rectal area directly, on sanitary napkins, tampons, or underwear. To estimate "talc-years", we multiplied applications-per year by years-used. Unconditional logistic regression, Wald statistics, likelihood-ratio tests, and polytomous logistic regression were used to calculate: adjusted odds ratios (OR) and 95% confidence intervals (CI), trends, effect-modification, and heterogeneity by ovarian cancer histologic subtype.

Results: Overall, genital talc use was associated with an OR (95% CI) of 1.33(1.16, 1.52) with a trend for increasing risk by talc-years. Women who used talc were more likely to be: older, heavier, asthma sufferers, and regular analgesic users—none of which were confounders. Dose-responses were more apparent for premenopausal women , especially non-smokers and those heavier, or postmenopausal users of menopausal hormones (HT). Subtypes of ovarian cancer more likely to be associated with talc included invasive serous and endometrioid tumors and borderline serous and mucinous tumors. Premenopausal women and postmenopausal HT-users with these subtypes who had accumulated >24 talc-years had ORs (95%CI) of 2.33(1.32, 4.12) and 2.57(1.51, 4.36), respectively.

Conclusion: Risks for epithelial ovarian cancer from genital talc use vary by histologic subtype, menopausal status at diagnosis, HT use, weight, and smoking. These observations suggest estrogen and/or prolactin may play a role via macrophage activity and inflammatory response to talc.

Introduction

In the 1960's, a link between talc and ovarian cancer was suggested by observations that some talc powders contained asbestos(1) and that asbestos placed intraperitoneally in animals transformed the single layer of the ovarian surface to a multilayered one with abnormal cells(2). A 1971 study found particles compatible with talc in human ovarian and uterine cancers(3). A 1982 case-control study was the first to link genital talc use with ovarian cancer(4). Dozens more followed confirming the association including some larger ones cited here (5-13). The most recent meta-analysis reported a summary OR and 95% confidence interval (CI) of 1.35(1.26, 1.46)(14). In 2006, the International Agency for Research on Cancer declared that talc used genitally is possibly carcinogenic(15). However, a study with null results from the Women's Health Initiative (WHI)(16) and accompanying editorial (17) cast new skepticism on the association. Here, we present data from combined phases of a case-control study of ovarian cancer involving more than 4000 women to provide fresh perspectives on this association.

Methods

Study population

Data come from three enrollment phases: 1 (1992-1997), 2 (1998-2002), and 3 (2003-2008). Papers we previously published related to talc include a detailed report from phase 1(7), data from phases 1 and 2 combined with Nurses' Health Study data(18), and phases 1-3 data combined with data from several participants in the Ovarian Cancer Association Consortium (OCAC) (19). This is the first detailed examination of talc data from the combined phases of our study.

Details regarding enrollment are described elsewhere(20). Briefly, 3957 women residing in Eastern Massachusetts and New Hampshire diagnosed with ovarian cancer between ages 18-80 were identified through tumor boards and registries. 874 cases were ineligible if they had died, moved outside study area, did not have a working telephone

number, or had a non-ovarian primary tumor. Of the remaining 3083 cases, 2203 (71%) were enrolled. Excluding 127 non-epithelial and 35 mixed mesodermal tumors, 2041 cases with epithelial tumors of ovarian, primary peritoneal, and Fallopian tube origin, including borderline malignancies (henceforth, epithelial ovarian cancer) were included. Pathology reports were reviewed and histologic subtype, grade, and stage recorded. Mixed epithelial ovarian cancer was classified as the predominant type. Undifferentiated, transitional cell, Fallopian tube, or primary peritoneal tumors were counted as serous(21). Other mixed epithelial (n=102), malignant Brenner (n=5), and unspecified epithelial tumors (n=27) were classified as other.

Controls were identified through random digit dialing, driver-license lists, and town-resident lists. Between 1992 and 1997, 420(72%) identified through random digit dialing and 102(51%) through lists agreed to participate. From 1998 to 2008, 4366 potential controls were identified using the lists, of whom 1426 (33%) were ineligible if they had died, moved, or were seriously ill or if they did not have a working telephone, speak English, or have ovaries. Of eligible controls, 1362(46%) declined to participate by phone or via 'opt-out' postcard and 1578(54%) were enrolled (2100 total). Controls were frequency matched to cases by 5-year age groups and region of residence.

Exposure assessment

Subjects were personally interviewed about potential ovarian cancer risk factors that occurred more than one year before diagnosis, for cases, and interview, for controls. Subjects were asked whether they "regularly" or "at least monthly" applied powder to: the genital or rectal area, sanitary napkins or tampons, underwear, or areas other than the genital-rectal area. Additional details included type of powder, age begun, years used, and applications per month. Lifetime exposure was estimated by multiplying frequency of applications per month by months used. This was divided by 360 (i.e. daily use coded as 30/month) to yield talc-years. To create categorical variables for talc-years, we chose cut points based on quartiles for exposed controls and rounded to the nearest integer.

Additionally, we asked participants if their partners dusted or sprayed powder to their genital or rectal areas. Condom and diaphragm use as potential sources of talc exposure were also recorded.

We calculated ovulatory cycles by subtracting age at menarche from age at last period, reduced this by time spent pregnant, breastfeeding, or using oral contraceptives, and dividing the remainder by each woman's average cycle length. Family history was defined as a mother or sister with ovarian or premenopausal breast cancer. Women who reported postmenopausal hormone use were classified as hormone therapy (HT) users and type(s) of HT was recorded. Participants completed a food-frequency questionnaire (FFQ)(22) from which grams of alcohol consumed per day were estimated.

Statistical methods

Unconditional logistic regression was used to model the odds ratio (OR) and 95% confidence interval (95% CI) adjusted first for matching factors (age, study center, and phase) and then fully by potential confounders. Likelihood ratio tests comparing models with and without interaction terms were used to test for effect modification. Tests for trend were based on the Wald statistic using continuous variables weighted by category midpoints with zero assigned as the exposure for non-users. Polytomous logistic regression was used to simultaneously estimate separate ORs and 95% CIs for genital talc use by histologic subtypes. Likelihood-ratio tests were used to calculate p-values for heterogeneity by comparing polytomous logistic regression models in which the talc association was held constant over case subgroups to models that allowed the association to differ between case subgroups(23). Analyses were performed using SAS v9.3 (SAS Institute, Cary, NC, USA) and polytomous logistic regression analyses were performed in Stata (StataCorp LP, College Station, TX, USA). Sensitivity analyses to assess the influence of exposure misclassification were performed with Excel using quantitative analysis methods described previously(24).

Ethical approval

Institutional review boards approved the study. All participants provided written informed consent.

Results

Genital use of talc, either alone or in combination with body use, was associated with elevated epithelial ovarian cancer risk (Table 1). Among women with no personal use, there was no increased risk with potential exposure from diaphragms, condoms, or partner use. Therefore, only those with personal genital talc exposure were classified as ever-users. Genital talc use was associated with an OR (95% CI) of 1.33 (1.16, 1.52) adjusted only for age, study center and phase. Most women reported using Johnson & Johnson's Baby Powder or Shower-to-Shower. Fourteen women who reported exclusive use of a cornstarch-based powder were considered unexposed. The average age women began using talc was 20.0 for cases and 19.8 for controls. Almost half of users were currently using or had only recently discontinued powder use at the reference date. Risk decreased with increased time since last use. The trend for frequency of use was significant, but the trend for years used was flat. Some subjects reported they used talc only seasonally, but our original questionnaire did not capture this detail. A question to capture months-per-year-used was added in 1998 and was available for 54% of cases and 56% of controls. Year-round use was the most common pattern, and more cases than controls used powder year-round. ORs for talc-years among those who reported months-per-year-used are shown as the next-to-final entry in Table 1. An OR of 1.49 (95% CI 1.06, 2.10) was associated with more than 20 talc-years (>7200 applications) and a dose-response. For subjects missing the seasonal-use variable, we assumed 12 months per year in calculating talc-years in the final entry in Table 1, as well as in subsequent tables and figures examining talc-years. Even with this imprecision, the trend remained, although the increase was less monotonic.

Figure 1 shows the proportions of cases and controls who used talc in the genital area by decade of birth and age at diagnosis or interview. In 13 of the 16 age-and-birth

categories, a greater proportion of cases used talc compared to controls. This suggests the association between genital use of talc and epithelial ovarian cancer is not confined to any particular age or birth cohort.

Powder users, both cases and controls, were more likely to be: older, heavier, asthma sufferers, and regular analgesic users (Table 2). By tests for interaction (column 3), the association was significantly greater for women who: were African American, had no personal history of breast cancer, had a tubal ligation or hysterectomy, were premenopausal, or were postmenopausal and used HT. The latter finding, together with the dose-response data, is illustrated in Figure 2. Among the HT users, 92% used estrogen (alone or in combination), 2% used progesterone alone, and 5% used creams or suppositories. Increased epithelial ovarian cancer risk with genital talc use was found in both women who had used estrogen alone or estrogen plus progesterone. Too few women used progesterone only HT or estrogen creams or suppositories to examine risk with talc use in these groups (data not shown). The median duration of HT use was 5 years. Subjects with <5 years of HT use had an overall OR (95%CI) for EOC risk with ever-use of talc on genitals of 2.93(1.86, 4.62). Subjects with ≥5 years of HT use had an OR (95%CI) that was slightly lower, 1.73(1.15, 2.62), but a clearer trend for increasing risk with talc-years was more apparent in the longer term HT users (data not shown). To explore the potential interaction between talc use and hysterectomy or tubal ligation, we restricted this analysis to subjects who had either or both procedures (Table 3). For premenopausal women, risk for EOC was increased in women who used talc before the procedure, while risk was elevated for use both before and after the procedure in postmenopausal women who used HT. No associations were seen in postmenopausal women who had not used HT. There were too few subjects who had used talc only after a hysterectomy or tubal ligation to permit reliable estimates of risk.

Returning to Table 2, we applied the convention that a variable may be a confounder if adjustment yields a 10% difference compared to the crude OR (or, in our study, compared to the OR of 1.33 adjusted for age, study center, and study phase). A 10% lower or greater change corresponds to an OR ≤1.20 or ≥1.46. As seen in the far right column, the OR of 1.33 for ovarian cancer risk was not materially changed after adjustment for any individual or all variables.

Because Figure 2 suggests that EOC risk with talc varies by menopausal status, we revisited the issue of interaction in eTable 1 in which subjects are stratified by menopausal status (http://links.lww.com/EDE/B2). Although few significant interactions were seen, categories for several variables revealed contrasting overall associations and/or clearer dose-responses (Figure 3). For premenopausal women, these included women: with a BMI>25; those who had breastfed; those who were not current smokers; and those who consumed more than 2.32 grams of alcohol per day. In addition, the association was stronger for both pre- and postmenopausal women who were least likely to have a genetic basis for their ovarian cancer, defined as women with no personal history of breast cancer, without a primary relative with either ovarian cancer or premenopausal breast cancer, and non-Jewish women (eTable 1; http://links.lww.com/EDE/B2). No important interactions were observed for postmenopausal women, except for weight and BMI, HT use, and the combined "genetic" variable.

Table 4 shows ORs stratified by menopausal status and histologic subtype of epithelial ovarian cancer. Overall, talc use increased risk for serous and endometrioid invasive and serous borderline tumors with the dose-response most apparent for serous invasive cancer. For premenopausal women, both the overall associations and dose-responses were stronger with serous invasive and serous borderline tumors. Premenopausal women also had an increased risk for mucinous borderline tumors at the highest quartile of talc use OR=2.28(1.23, 4.26) and a dose-response. For postmenopausal women, dose-responses were strongest for women with invasive serous

and endometrioid tumors. Talc use was not associated with clear cell or mucinous invasive epithelial ovarian cancer regardless of menopausal status. The ORs and dose-responses for the combined histologic subtypes relevant to pre- and postmenopausal women are shown in Table 5. Except for a few categories, these were not materially different than those illustrated in Figure 2. However, notably, premenopausal women and postmenopausal HT-users with the relevant subtypes who had accumulated >24 talc-years had ORs (95% CI) of 2.33(1.32, 4.12) and 2.57(1.51, 4.36), respectively.

Discussion

We analyzed case-control data collected over 16 years on talc use and epithelial ovarian cancer risk to address issues related to definition of the exposure, bias and confounding, effect modification, histologic heterogeneity, and dose-response. Talc used regularly in the genital area was associated with a 33% increase in ovarian cancer risk overall while no apparent risk was associated with talc used only in non-genital areas. Our results are consistent with a recent pooled analysis from the OCAC which reported that use of powder on genitals is associated with a 24% increased risk and no effect of non-genital use of talc (19). There was general agreement on risk by histologic type of epithelial ovarian cancer except that OCAC found an association with clear cell cancer and we did not. The findings from OCAC and our study contrast with null results from the WHI cohort analysis (17) raising the issue of recall bias in case-control studies.

Addressing recall bias, we conducted a sensitivity analysis that assumed truly non-exposed cases and controls were accurately classified as unexposed (i.e. specificity 99%) and truly exposed cases were also correctly classified (sensitivity 99%). The OR of 1.33 in our study would be nullified if the sensitivity of correctly classified controls fell to 82% or 18% misclassification. Unfortunately, there are no external records to validate talc use reported by study participants to assess whether this degree of misclassification is reasonable. Somewhat analogous to talc and ovarian cancer is alcohol use and breast

cancer. Nurses' Health Study investigators examined the latter association both with prospective data collected at baseline and retrospective data obtained by re-surveying subjects after diagnosis(25). They found an (age adjusted) OR for breast cancer of 1.42 associated with 30 or more grams of alcohol/day relative to non-drinkers from the prospective data compared with 1.33 from the retrospective data. This change between two analyses would occur if the sensitivity of controls correctly recalling alcohol use dropped to 91% (or 9% misclassification). This suggests some degree of misclassification in retrospective data but not as great as the 18% required to nullify the association between use of talc on genitals and ovarian cancer risk in our study. No comparable study on talc comparing results from prospective versus retrospective data has been performed. However, several observations are incompatible with the possibility that recall bias explains the association: 1) ORs are generally lower in studies which asked about "ever use" of talc (5,8,11) compared to those that specified regular use (6,7,9,12,13) whereas higher ORs would be expected if cases are more likely to recall limited ever-use; 2) no association with non-genital talc use; 3) risk varies by histologic type; 4) the association is stronger in premenopausal women who are closer in time to talc use and less likely to have forgotten it; and 5) ORs from recent studies (11, 13) are lower than those from earlier ones (6, 7) whereas increasing publicity about the association over time might lead to greater recall bias and higher ORs in more recent studies. Related arguments that cases initiate talc use because of treatment of ovarian cancer or early symptoms of disease also lack merit because we censored exposures 1 year prior to the date of diagnosis and most talc-users began the habit around age 20—a decade or more before the ovarian cancer diagnosis.

Whether the association is a result of confounding must also be addressed. A 1998 paper identified BMI, smoking, and alcohol use as potential correlates of talc use in the general population (26). In our study, powder users were more likely to be: older, from

more urban/suburban areas, heavier, asthma sufferers, and regular analgesics users. However, none of these or other Table 2 variables altered the overall association by more than 10%, providing no indication of confounding. Talc use was also greater in African Americans and notably associated with a high, albeit imprecise, OR (and 95% CI) of 5.08 (1.32, 19.6). This finding clearly requires further study.

The observation that talc users, both case and control subjects, were more likely to say they had asthma has not been previously reported. The link between powder use and asthma may not be fully appreciated from Table 2 since women who used talc as a body powder but not to the genital area were classified as non-exposed. Making no body or genital exposure the non-exposed referent group and asthma the outcome, the ORs (and 95% CI) for asthma for body exposure to talc is 1.27 (0.80, 2.03) for cases and 1.02 (0.66, 1.57) for controls. The comparable OR for genital use of talc with or without body use is 1.48 (1.00, 2.18) for cases and 1.45 (1.00, 2.10) for controls. 60 of 85 cases (70%) with asthma and 57 of 89 (64%) controls reported that talc use predated asthma onset. Although chance must be considered a possible explanation for this novel finding, talc is a cause of occupational asthma(27) and respiratory distress has been reported in infants after talc was accidentally inhaled(28). That asthma may be associated with use of talc is important not only because of health consequences on its own, but also because it may shed light on biologic mechanisms potentially relevant to the talc and ovarian cancer association.

Although we found no evidence of confounding, we did find several examples of effect modification of the association between talc and epithelial ovarian cancer. Overall, the association was greater in women with no personal history of breast cancer, those who had a tubal ligation or hysterectomy, in premenopausal women, and postmenopausal women who had used HT. Among these factors, perhaps the most important is effect modification of the association by menopausal status and menopausal HT.

Apparent lack of an elevated risk for epithelial ovarian cancer from talc use in postmenopausal women without HT use has not been reported previously. Explanations might include: that there is no association with talc use in the absence of endogenous or exogenous estrogen, fading memory of past exposures, women who will develop ovarian cancer from talc use leave the at risk pool before they reach menopause, or more complex interactions with multiple risk factors and gene-environment interactions. Of possible relevance, Moorman et al. observed that reproductive events that clearly affect risk in premenopausal women may not affect risk to the same degree in postmenopausal women(29). Whatever the explanation, our observation challenges the relevance of the WHI study to the ovarian cancer/talc association since only postmenopausal women were enrolled in WHI and HT use was examined only as a confounder, not an effect modifier(16). Further study will be necessary to clarify the role that talc may play in postmenopausal women who did not use HT with a focus on those factors that may increase endogenous estrogen, such as greater BMI.

That the association is more apparent in premenopausal women and in postmenopausal women who used hormonal therapy suggests that estrogen plays a role in the association. In talc inhalation studies conducted by the National Toxicology Program, only female rats developed lung tumors(30). Literature on airway inflammation from particulates is also relevant. Citing evidence that asthma may be exacerbated during pregnancy, Zhang et al. postulated this may be due to an effect of estrogen on macrophage activity and inflammatory response to particulates normally considered inert, like titanium dioxide (TiO2)(31). Their in-vivo studies demonstrated that macrophages from pregnant mice transplanted to non-pregnant recipients conferred an inflammatory phenotype in response to TiO2. Such studies should be repeated with talc, another particulate considered "inert."

An exploratory analysis of other potential effect modifiers led to several other observations that may have biologic relevance. The overall associations and dose-

responses were "stronger" for premenopausal women who: had a greater BMI, had breastfed, were not current smokers, and consumed alcohol (Figure 3). Due to the large number of associations tested, chance must be the first explanation considered. However, a common denominator could be prolactin since its levels are higher in women who: have greater BMI(32), breastfed(33), do not currently smoke(34), consume alcohol(35), and are postmenopausal and use HT(36). Like estrogen, prolactin may have multiple effects on immune cells, especially monocytes and macrophages(37) whose role in scavenging talc in tissue is described(38). These observations provide a framework for talc carcinogenicity in EOC involving chronic inflammation(9).

Biologic credibility of the talc/EOC association is enhanced by persuasive evidence that inert particles the size of talc, present in the vagina, can migrate to the upper genital tract. In a technique called hysterosalpingoscintigraphy, technetium-labeled albumen microspheres are placed in the vagina and their migration to the upper tract confirmed using serial scintograms(39). The microspheres are 5 to 40 microns in diameter—a range which includes the size of sperm and talc. Migration from the vagina is the obvious explanation for why talc can be found in diseased (and some normal) ovaries(3). Unfortunately, no epidemiologic study of epithelial ovarian cancer and talc has taken the opportunity to determine whether talc can actually be found in tissues removed at surgery and correlated with exposure to talc. A clue to talc's presence is birefringent particles found when slides are examined under polarized-light microscopy. Although confirmation that the material is actually talc requires scanning electron microscopy and X-ray dispersion spectroscopy, presence of birefringence is a practical screening technique as illustrated by a case report of a woman with ovarian cancer and long-term talc use who had talc in her pelvic lymph nodes first suggested by birefringence(40).

There are inherent limitations quantifying a dose-response due to a lack of metrics for how much talc is in an "application," how much enters the vagina, and how much

reaches the upper genital tract where, presumably, any deleterious effect is mediated. This may account for the failure to identify a dose-response in many papers on talc and ovarian cancer. Our 1999 study (7) suggested that adjusting total applications by whether the genital tract was "open" (i.e. excluding use after a tubal ligation or hysterectomy and examining use during times when ovulation was occurring) yielded significant dose-responses. Mills et al. found a dose-response by frequency of use(10). Wu, looking at all types of body use, found a dose-response with estimated applications(12). Merritt reported a significant trend in risk for invasive serous ovarian cancer with years of talc use(11). The recent OCAC analysis reported no trend with increasing lifetime applications *when restricted to talc users*(19). However, an increase in risk with increasing applications was found for non-mucinous epithelial ovarian cancer *when non-users were included*. Virtually all papers that have looked at dose-response for talc and epithelial ovarian cancer risk have included non-users in the trend analysis. In our paper, we calculated talc-years and showed that, overall, there is a significant trend for epithelial ovarian cancer risk and talc-years when non-users are included, and the trend is even more apparent in premenopausal women with certain epithelial ovarian cancer subtypes.

In summary, this study on talc and epithelial ovarian cancer has contributed the following perspectives, some new, regarding this association:

1) Overall, there is an association between genital talc use and EOC and a significant trend with increasing "talc-years" of use.
2) Among many epidemiologic variables, no confounders for the association were identified.
3) Talc users, both cases and controls, were more likely to report a medical history of asthma.
4) The talc/epithelial ovarian cancer association was largely confined to premenopausal women and postmenopausal women who used HT. Other potential effect modifiers in premenopausal women included BMI,

breastfeeding, current smoking, or alcohol use. These observations may suggest a role for estrogen and/or prolactin, both known to affect macrophage function and inflammatory response.

5) Histologic subtypes of epithelial ovarian cancer more likely to be associated with talc include serous and mucinous borderline tumors and invasive serous and endometrioid tumors.
6) For epithelial ovarian cancer categories based on certain effect modifiers or histologic subtypes, stronger overall associations and dose-responses were observed.
7) The association may be stronger in African Americans.

An editorial(17) accompanying the WHI study(16) noted that "several case-control studies have reported associations between talc use and ovarian cancer risk" and "no epidemiologic studies have demonstrated a dose-response" (page 2). We believe these appraisals understate the epidemiologic evidence. There have been dozens of case-control studies and several have, in fact, found a dose-response. The editorial further notes that "it does not seem likely that additional conventional epidemiologic studies will strengthen the evidence for or against talc carcinogenicity" (page 2). We believe the observations made here present a good case for talc carcinogenicity and that re-analyses of existing data from already published studies might provide confirmatory evidence. To encourage consolidation of data, we have provided a copy of the "raw" and derived variables examined in our study to NCI dbGaP (available here: http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001034.v1.p1) as well as the SAS and Stata programs used in this analysis (eAppendix 1; http://links.lww.com/EDE/B2).

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Figure 1. Proportions of cases and controls who ever used talc on genitals in categories by decade of birth and reference age.

Figure 2. Associations between use of talc on genitals (never/ever and quartiles of talc-years) and ovarian cancer by menopausal status and postmenopausal hormone therapy.

Figure 3. Variables modifying the talc association in premenopausal women. [a] p-heterogeneity from likelihood ratio tests comparing a model with ever/never talc use and the effect modifier to a model with these plus the interacton term between them. [b] p-heterogeneity from likelihood ratio tests comparing a model with indicators for each quartile of talc-years and the effect modifier to a model with these plus their interacton terms.

Table 1. Type, timing, and duration of genital talc use.

	Control subjects N (%)	Case subjects N (%)	Adjusted[a] OR (95% CI)
Personal use			
None	1099 (52)	1001 (49)	1.00 (referent)
Body use only	452 (22)	398 (20)	0.99 (0.84, 1.16)
Genital use only	74 (4)	94 (5)	1.42 (1.04, 1.96)
Body and genital use	475 (23)	548 (27)	1.30 (1.12, 1.52)
Potential exposure in women with no personal use			
None	447 (41)	461 (46)	1.00 (referent)
Diaphragm only	207 (19)	155 (15)	0.73 (0.57, 0.93)
Condoms, with or without diaphragm	367 (33)	308 (31)	0.82 (0.66, 1.01)
Partner use, with or without diaphragm or condoms	78 (7)	77 (8)	0.96 (0.68, 1.35)
Any genital powder use			
No	1551 (74)	1399 (69)	1.00 (referent)
Yes	549 (26)	642 (31)	1.33 (1.16, 1.52)
Type of genital powder used			
No genital use	1542 (73)	1394 (68)	1.00 (referent)
Cornstarch use only	9 (<1)	5 (<1)	0.58 (0.19, 1.74)
Johnson and Johnson Baby Powder or Shower to Shower	316 (15)	363 (18)	1.30 (1.10, 1.54)
Other brand(s)	233 (11)	279 (14)	1.35 (1.12, 1.64)
Age first used genital powder[b]			
Never used	1551 (74)	1399 (69)	1.00 (referent)
<20	343 (16)	363 (18)	1.19 (1.01, 1.41)
20-29	122 (6)	183 (9)	1.71 (1.34, 2.17)
≥30	76 (4)	87 (4)	1.31 (0.95, 1.80)
Time since exposure ended			
No genital use	1551 (74)	1399 (69)	1.00 (referent)
≥35 years	51 (2)	52 (3)	1.18 (0.79, 1.75)
25-34 years	81 (4)	88 (4)	1.24 (0.91, 1.70)
15-24 years	72 (3)	82 (4)	1.30 (0.94, 1.80)
5-14 years	79 (4)	95 (5)	1.36 (1.00, 1.85)
Currently using or recently stopped	255 (12)	314 (15)	1.38 (1.15, 1.65)
p-trend			<0.0001
Frequency of use			
No genital use	1551 (74)	1399 (69)	1.00 (referent)
1-7 days per month	220 (11)	227 (11)	1.17 (0.96, 1.44)
8-29 days per month	110 (5)	133 (7)	1.37 (1.05, 1.78)
≥30 days per month	205 (10)	267 (13)	1.46 (1.20, 1.78)
p-trend			<0.0001
Years used			
Never used	1551 (74)	1399 (69)	1.00 (referent)
<8	133 (6)	152 (8)	1.31 (1.03, 1.68)
8-19	126 (6)	145 (7)	1.31 (1.02, 1.68)
20-35	147 (7)	178 (9)	1.35 (1.07, 1.70)
>35	129 (6)	152 (7)	1.33 (1.03, 1.71)
p-trend			0.002
Months per year of use			
No genital use	1551 (83)	1399 (80)	1.00 (referent)
1-3 months per year	61 (3)	60 (3)	1.11 (0.77, 1.61)
4-11 months per year	55 (3)	56 (3)	1.13 (0.77, 1.66)
12 months per year	193 (10)	229 (13)	1.35 (1.09, 1.67)
p-trend			0.006
Total genital talc applications (apps) among only those who reported months per year of use[c]			
No genital use	1551 (83)	1399 (80)	1.00 (referent)
≤360 apps (equivalent to 1 year of daily use)	106 (6)	103 (6)	1.10 (0.83, 1.47)
361-1800 apps (equivalent to >1-5 years of daily use)	79 (4)	96 (5)	1.38 (1.01, 1.88)
1801-7200 apps (equivalent to >5-20 years of daily use)	61 (3)	63 (4)	1.16 (0.80, 1.66)
>7200 apps (equivalent to >20 years of daily use)	63 (3)	83 (5)	1.49 (1.06, 2.10)
p-trend			0.02
Total genital talc applications among all (assuming 12 months/year when missing months per year of use)			
No genital use	1551 (74)	1399 (69)	1.00 (referent)
≤360 apps (equivalent to 1 year of daily use)	138 (7)	138 (7)	1.15 (0.89, 1.47)
361-1800 apps (equivalent to >1-5 years of daily use)	124 (6)	148 (7)	1.36 (1.06, 1.75)
1801-7200 apps (equivalent to >5-20 years of daily use)	124 (6)	156 (8)	1.41 (1.10, 1.80)
>7200 apps (equivalent to >20 years of daily use)	149 (7)	185 (9)	1.39 (1.11, 1.75)
p-trend			0.003

[a]Adjusted only for the study matching factors: reference age, study center and study phase.
[b]9 cases and 9 controls reported they knew that talc had been used on them in infancy so their age at exposure was recorded as 1.
[c]Excludes talc users from phase 1 and part of phase 2 because months/year of use was not collected.

Table 2. Illustrating potential effect modification and confounding.

	Controls		Cases				
	No genital talc use N (%)	Any genital talc use N (%)	No genital talc use N (%)	Any genital talc use N (%)	Stratum specific OR (95% CI)[a] for genital talc use	p-int[b]	OR (95% CI) for genital talc use adjusted[c]
Age							
<50	670 (80)	165 (20)	600 (74)	211 (26)	1.42 (1.13, 1.80)	0.63	1.30 (1.13, 1.49)[d]
50-64	599 (68)	278 (32)	541 (64)	308 (36)	1.25 (1.03, 1.53)		
≥65	282 (73)	106 (27)	258 (68)	123 (32)	1.35 (0.98, 1.85)		
Study center							
New Hampshire	319 (82)	72 (18)	316 (74)	109 (26)	1.52 (1.08, 2.14)	0.30	1.31 (1.15, 1.50)[e]
Massachusetts	1232 (72)	477 (28)	1083 (67)	533 (33)	1.29 (1.11, 1.50)		
Study phase							
1	430 (82)	92 (18)	409 (73)	149 (27)	1.71 (1.27, 2.30)	0.12	1.33 (1.16, 1.52)[f]
2	519 (72)	202 (28)	448 (68)	210 (32)	1.23 (0.97, 1.55)		
3	602 (70)	255 (30)	542 (66)	283 (34)	1.25 (1.02, 1.54)		
Race							
White	1500 (74)	531 (26)	1321 (68)	612 (32)	1.35 (1.17, 1.55)	0.002	1.33 (1.16, 1.53)
African American	17 (74)	6 (26)	16 (46)	19 (54)	5.08 (1.32, 19.6)		
Hispanic	27 (82)	6 (18)	25 (81)	6 (19)	1.10 (0.30, 4.12)		
Asian	5 (50)	5 (50)	34 (94)	2 (6)	0.04 (0.01, 0.34)		
Other	2 (67%)	1 (33)	3 (50)	3 (50)	--		
Body mass index							
<24.9	798 (76)	251 (24)	727 (72)	284 (28)	1.25 (1.03, 1.53)	0.59	1.32 (1.15, 1.51)
≥24.9	753 (72)	298 (28)	672 (65)	358 (35)	1.38 (1.14, 1.67)		
Height (m)							
<1.63	755 (73)	283 (27)	689 (68)	325 (32)	1.28 (1.06, 1.56)	0.71	1.32 (1.16, 1.52)
≥1.63	795 (75)	266 (25.)	710 (69)	317 (31)	1.37 (1.13, 1.66)		
Weight (lbs)							
<148	799 (77)	241 (23)	727 (73)	272 (27)	1.24 (1.01, 1.52)	0.58	1.32 (1.15, 1.51)
≥148	745 (71)	307 (29)	670 (64)	370 (36)	1.38 (1.15, 1.66)		
Parity							
Nulliparous	284 (75)	94 (25)	455 (70)	195 (30)	1.28 (0.96, 1.71)	0.71	1.33 (1.15, 1.52)
Parous	1267 (74)	455 (26)	944 (68)	447 (32)	1.34 (1.15, 1.57)		
Ever breastfed							
No	781 (72)	296 (28)	953 (69)	430 (31)	1.21 (1.01, 1.45)	0.16	1.30 (1.13, 1.50)
Yes	770 (75)	253 (25)	446 (68)	212 (32)	1.48 (1.19, 1.85)		
OC use							
Never or <3 months	559 (73)	207 (27)	672 (69)	302 (31)	1.25 (1.01, 1.55)	0.38	1.32 (1.15, 1.51)
≥3 months	992 (74)	342 (26)	727 (68)	340 (32)	1.39 (1.16, 1.67)		

Table 2. Illustrating potential effect modification and confounding. (continued)

	Controls		Cases				
	No genital talc use N (%)	Any genital talc use N (%)	No genital talc use N (%)	Any genital talc use N (%)	Stratum specific OR (95% CI) [a] for genital talc use	p-int[b]	OR (95% CI) for genital talc use adjusted[c]
IUD use							
No	1300 (74)	447 (26)	1203 (69)	547 (31)	1.35 (1.16, 1.56)	0.59	1.33 (1.16, 1.52)
Yes	251 (71)	102 (29)	196 (67)	95 (33)	1.20 (0.85, 1.70)		
Ovulatory cycles							
<366	748 (78)	214 (22)	542 (74)	191 (26)	1.28 (1.02, 1.61)	0.76	1.31 (1.14, 1.52)
≥366	680 (71)	281 (29)	733 (65)	402 (35)	1.37 (1.13, 1.65)		
Endometriosis or painful periods							
No	1006 (74)	345 (26)	814 (70)	351 (30)	1.29 (1.08, 1.55)	0.77	1.31 (1.14, 1.50)
Yes	545 (73)	204 (27)	585 (67)	291 (33)	1.35 (1.09, 1.67)		
Jewish ethnicity							
No	1455 (74)	518 (26)	1277 (69)	585 (31)	1.33 (1.15, 1.53)	0.72	1.33 (1.16, 1.52)
Yes	96 (76)	31 (24)	122 (68)	57 (32)	1.39 (0.83, 2.33)		
Family history[d]							
No	1446 (74)	510 (26)	1267 (68)	585 (32)	1.34 (1.16, 1.55)	0.61	1.33 (1.16, 1.52)
Yes	105 (73)	39 (27)	132 (70)	57 (30)	1.19 (0.73, 1.93)		
Personal history of breast cancer							
No	1498 (74)	519 (26)	1299 (68)	606 (32)	1.38 (1.20, 1.59)	0.01	1.33 (1.16, 1.53)
Yes	53 (64)	30 (36)	100 (74)	36 (26)	0.67 (0.37, 1.22)		
Hysterectomy or tubal ligation							
No	1135 (74)	401 (26)	1134 (70)	480 (30)	1.22 (1.04, 1.43)	0.02	1.34 (1.16, 1.53)
Yes	416 (74)	148 (26)	265 (62)	162 (38)	1.73 (1.31, 2.27)		
Menopausal status and hormone therapy (HT)							
Premenopausal	735 (79)	197 (21)	653 (73)	247 (27)	1.41 (1.13, 1.75)	<0.001	1.33 (1.16, 1.53)
Postmenopausal, no HT	507 (69)	230 (31)	549 (70)	238 (30)	0.97 (0.78, 1.20)		
Postmenopausal, HT	309 (72)	122 (28)	197 (56)	157 (44)	2.21 (1.63, 3.00)		
Current smoking							
No	1332 (74)	473 (26)	1149 (68)	538 (32)	1.35 (1.16, 1.56)	0.60	1.32 (1.16, 1.52)
Yes	219 (74)	76 (26)	250 (71)	104 (29)	1.19 (0.84, 1.69)		
Ever smoked							
No	759 (75)	248 (25)	669 (70)	291 (30)	1.34 (1.10, 1.64)	0.72	1.32 (1.16, 1.52)
Yes	792 (72)	301 (28)	730 (68)	351 (32)	1.31 (1.09, 1.58)		
Asthma							
No	1442 (75)	492 (25)	1310 (69)	586 (31)	1.34 (1.16, 1.55)	0.70	1.33 (1.16, 1.52)
Yes	109 (66)	57 (34)	89 (61)	56 (39)	1.25 (0.78, 2.01)		
Alcohol (grams per day)							
≤2.32 grams	753 (74)	269 (26)	738 (70)	311 (30)	1.19 (0.98, 1.45)	0.29	1.30 (1.13, 1.50)
>2.32 grams	763 (75)	259 (25)	623 (68)	291 (32)	1.43 (1.17, 1.75)		

	Controls		Cases				
	No genital talc use N (%)	Any genital talc use N (%)	No genital talc use N (%)	Any genital talc use N (%)	Stratum specific OR (95% CI) [a] for genital talc use	p-int[b]	OR (95% CI) for genital talc use adjusted[c]
Any acetaminophen use							
No	1190 (76)	373 (24)	1076 (71)	431 (29)	1.30 (1.10, 1.53)	0.83	1.32 (1.15, 1.52)
Yes	361 (67)	176 (33)	323 (60)	211 (40)	1.41 (1.09, 1.82)		
Any aspirin or ibuprofen use							
No	936 (77)	285 (23)	901 (71)	361 (29)	1.32 (1.10, 1.59)	0.94	1.34 (1.17, 1.53)
Yes	615 (70)	264 (30)	498 (64)	281 (36)	1.38 (1.11, 1.68)		
Adjusted for all variables	1551	549	1399	642	--	--	1.32 (1.15, 1.53)

[a] Adjusted for reference age (continuous), study center and study phase.
[b] p for interaction from likelihood ratio tests comparing models with main effects and interaction terms to models with main effects only.
[c] Adjusted for reference age (continuous), study center, study phase and each variable listed (individually). BMI, height, weight and ovulatory cycles were adjusted for with indicators for quartiles and parity (nulliparous, 1, 2, ≥2), breast feeding (never, <4, 4-9, 10-19, >19 months), and OC use (never, <23, 23-49, 50-96, >96 months) were adjusted for with indicators for categories
[d] Adjusted for reference age only.
[e] Adjusted for reference age and study center.
[f] Adjusted for reference age, study center and study phase.
[g] Family history of ovarian or early onset breast cancer in a mother or sister.

Table 3. Effect of tubal ligation and hysterectomy by menopausal status and hormone therapy on association between genital talc use and ovarian cancer.

Genital talc use among women who had a hysterectomy or tubal ligation[b]	Premenopausal			Postmenopausal, never used HT			Postmenopausal, ever used HT		
	Controls N (%)	Cases N (%)	Adjusted[a] OR (95% CI)	Controls N (%)	Cases N (%)	Adjusted[a] OR (95% CI)	Controls N (%)	Cases N (%)	Adjusted[a] OR (95% CI)
Never used	147 (79)	94 (71)	1.00 (referent)	139 (67)	113 (67)	1.00 (referent)	130 (77)	58 (48)	1.00 (referent)
Used both before and after	26 (14)	17 (13)	0.99 (0.48, 2.06)	45 (22)	36 (21)	1.00 (0.58, 1.72)	21 (13)	40 (33)	5.85 (2.88, 11.9)
Used before only	10 (5)	20 (15)	4.40 (1.73, 11.2)	20 (10)	16 (10)	0.99 (0.46, 2.10)	12 (7)	18 (15)	3.49 (1.39, 8.75)
Used after only	3 (2)	1 (1)	0.33 (0.03, 3.60)	3 (1)	4 (2)	1.66 (0.34, 8.21)	5 (3)	5 (4)	2.11 (0.49, 9.17)

[a] Adjusted for reference age (continuous), study center, study phase (3, 4, 5), parity (nulliparous, 1, 2, ≥2), breast feeding (never, <4, 4-9, 10-19, >19 months), OC use (never, <23, 23-49, 50-96, >96 months), IUD (never, ever), endometriosis or painful periods, personal history of breast cancer, Jewish ethnicity, tubal ligation, and BMI (<22.2, 22.2-24.8, 24.9-28.6, >28.6).

[b] The median ages for tubal ligation and hysterectomy, respectively, were 34 and 39 for cases and 34 and 40 for controls.

Table 4. Genital talc applications by histologic type and menopausal status.

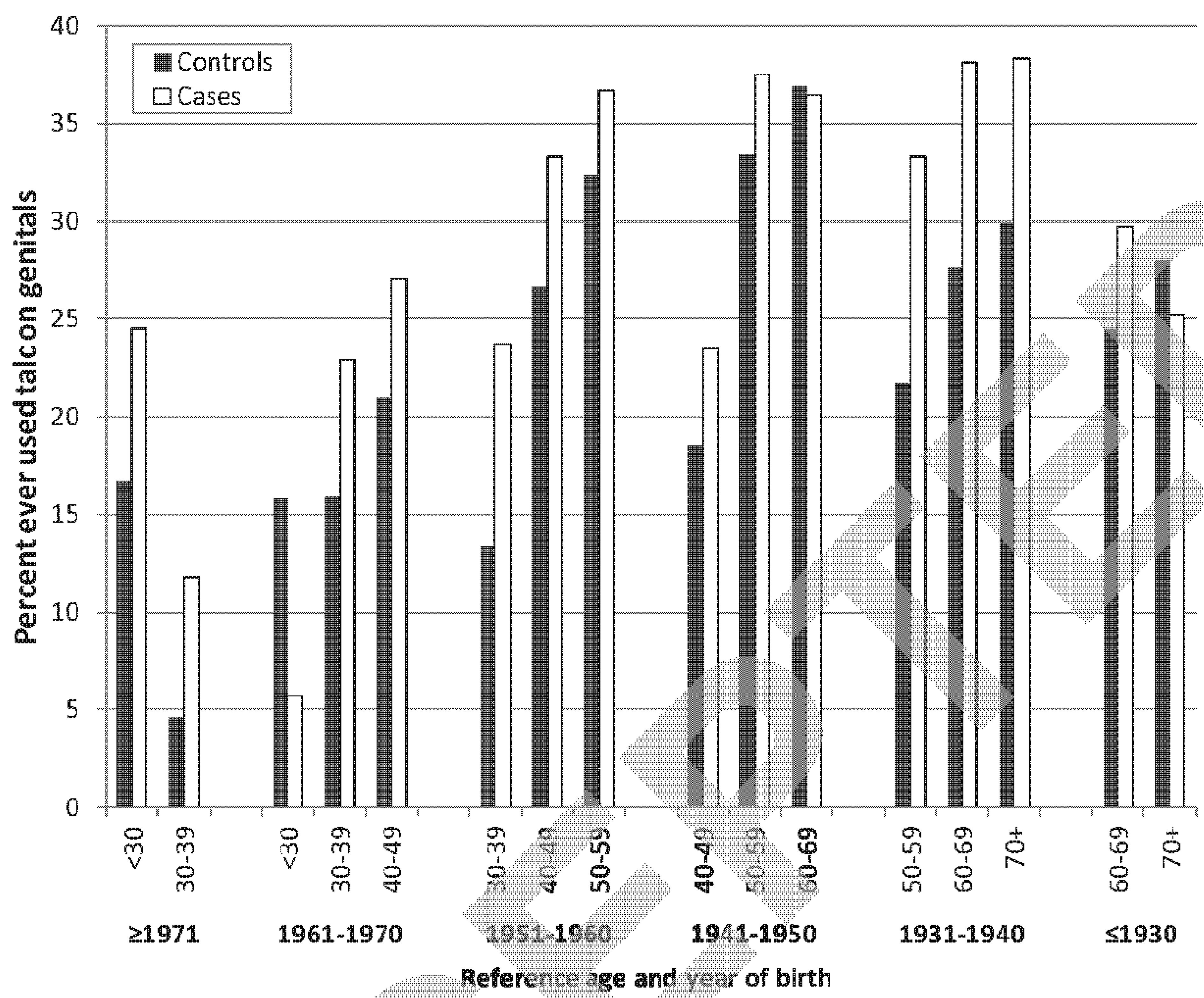
Characteristic	Controls	Serous invasive		Mucinous invasive		Endometrioid invasive		Clear cell invasive			Serous borderline		Mucinous borderline		P-het
	%	Cases %	Adjusted[a] OR (95% CI)	Cases %	Adjusted[a] OR (95% CI)	Cases %	Adjusted[a] OR (95% CI)	Cases %	Adjusted[a] OR (95% CI)	P-het	Cases %	Adjusted[a] OR (95% CI)	Cases %	Adjusted[a] OR (95% CI)	
All women	N=2100	N=968		N=95		N=327		N=114			N=250		N=147		
No use	74%	65%	1.00	78%	1.00	67%	1.00	74%	1.00	0.13	70%	1.00	78%	1.00	0.20
Any use	26%	35%	1.42 (1.19, 1.69)	22%	0.87 (0.53, 1.44)	33%	1.38 (1.06, 1.80)	26%	1.01 (0.65, 1.57)		30%	1.40 (1.03, 1.90)	22%	1.02 (0.67, 1.54)	
No genital use	74%	66%	1.00	78%	1.00	67%	1.00	74%	1.00		70%	1.00	78%	1.00	
≤1 talc-year	7%	7%	1.30 (0.94, 1.79)	8%	1.30 (0.60, 2.82)	8%	1.30 (0.81, 2.07)	6%	0.94 (0.42, 2.14)		8%	1.38 (0.80, 2.36)	2%	0.33 (0.10, 1.06)	
>1-5 talc-years	6%	7%	1.45 (1.05, 2.01)	3%	0.57 (0.18, 1.85)	8%	1.54 (0.96, 2.46)	7%	1.44 (0.66, 3.13)		8%	1.72 (1.02, 2.89)	6%	1.31 (0.63, 2.71)	
>5-24 talc-years	7%	9%	1.33 (0.99, 1.79)	8%	1.15 (0.54, 2.46)	8%	1.14 (0.72, 1.80)	5%	0.63 (0.27, 1.51)		8%	1.18 (0.69, 2.00)	11%	1.64 (0.92, 2.92)	
>24 talc-years	6%	11%	1.54 (1.15, 2.07)	2%	0.38 (0.09, 1.60)	9%	1.67 (1.06, 2.63)	8%	1.35 (0.64, 2.84)		6%	1.55 (0.87, 2.77)	3%	0.84 (0.32, 2.16)	
p-trend			0.003		0.24		0.04		0.64	0.16		0.16		0.76	0.55
Premenopausal	N=932	N=282		N=51		N=177		N=56			N=175		N=108		
No use	79%	70%	1.00	78%	1.00	70%	1.00	79%	1.00	0.49	72%	1.00	78%	1.00	0.44
Any use	21%	30%	1.43 (1.04, 1.98)	22%	1.04 (0.52, 2.10)	30%	1.34 (0.91, 1.96)	21%	0.87 (0.44, 1.75)		28%	1.56 (1.06, 2.31)	22%	1.25 (0.75, 2.06)	
No genital use	79%	70%	1.00	78%	1.00	71%	1.00	79%	1.00		72%	1.00	78%	1.00	
≤1 talc-year	7%	5%	0.71 (0.38, 1.34)	14%	1.81 (0.75, 4.37)	9%	1.13 (0.60, 2.11)	4%	0.33 (0.08, 1.47)		9%	1.51 (0.82, 2.80)	1%	0.13 (0.02, 0.99)	
>1-5 talc-years	5%	7%	1.71 (0.94, 3.12)	4%	1.01 (0.23, 4.42)	7%	1.58 (0.77, 3.27)	9%	2.41 (0.83, 7.01)		7%	1.58 (0.78, 3.21)	6%	1.57 (0.66, 3.74)	
>5 talc-years	9%	18%	1.85 (1.21, 2.80)	4%	0.44 (0.10, 1.90)	13%	1.33 (0.77, 2.31)	9%	0.87 (0.32, 2.38)		12%	1.66 (0.96, 2.88)	15%	2.28 (1.23, 4.26)	
p-trend			0.003		0.24		0.34		0.88	0.06		0.09		0.005	0.28
Postmenopausal	N=1168	N=686		N=44		N=150		N=58			N=75		N=39		
No use	70%	63%	1.00	77%	1.00	63%	1.00	69%	1.00	0.29	65%	1.00	77%	1.00	0.43
Any use	30%	37%	1.36 (1.10, 1.67)	23%	0.70 (0.34, 1.46)	37%	1.36 (0.94, 1.97)	31%	1.10 (0.61, 1.99)		35%	1.15 (0.69, 1.91)	23%	0.80 (0.37, 1.75)	
No genital use	70%	64%	1.00	77%	1.00	63%	1.00	69%	1.00		65%	1.00	77%	1.00	
≤5 talc-years	13%	15%	1.44 (1.07, 1.93)	5%	0.34 (0.08, 1.46)	15%	1.39 (0.82, 2.33)	14%	1.32 (0.58, 2.99)		16%	1.40 (0.70, 2.79)	10%	1.24 (0.41, 3.77)	
>5-24 talc-years	8%	9%	1.19 (0.83, 1.71)	14%	1.67 (0.66, 4.20)	9%	1.15 (0.61, 2.19)	3%	0.39 (0.09, 1.69)		8%	1.11 (0.45, 2.73)	8%	1.03 (0.30, 3.55)	
>24 talc-years	9%	12%	1.33 (0.96, 1.85)	5%	0.45 (0.10, 1.91)	13%	1.60 (0.93, 2.77)	14%	1.59 (0.70, 3.60)		11%	0.99 (0.44, 2.21)	5%	0.39 (0.09, 1.76)	
p-trend			0.13		0.49		0.12		0.44	0.58		0.91		0.23	0.29

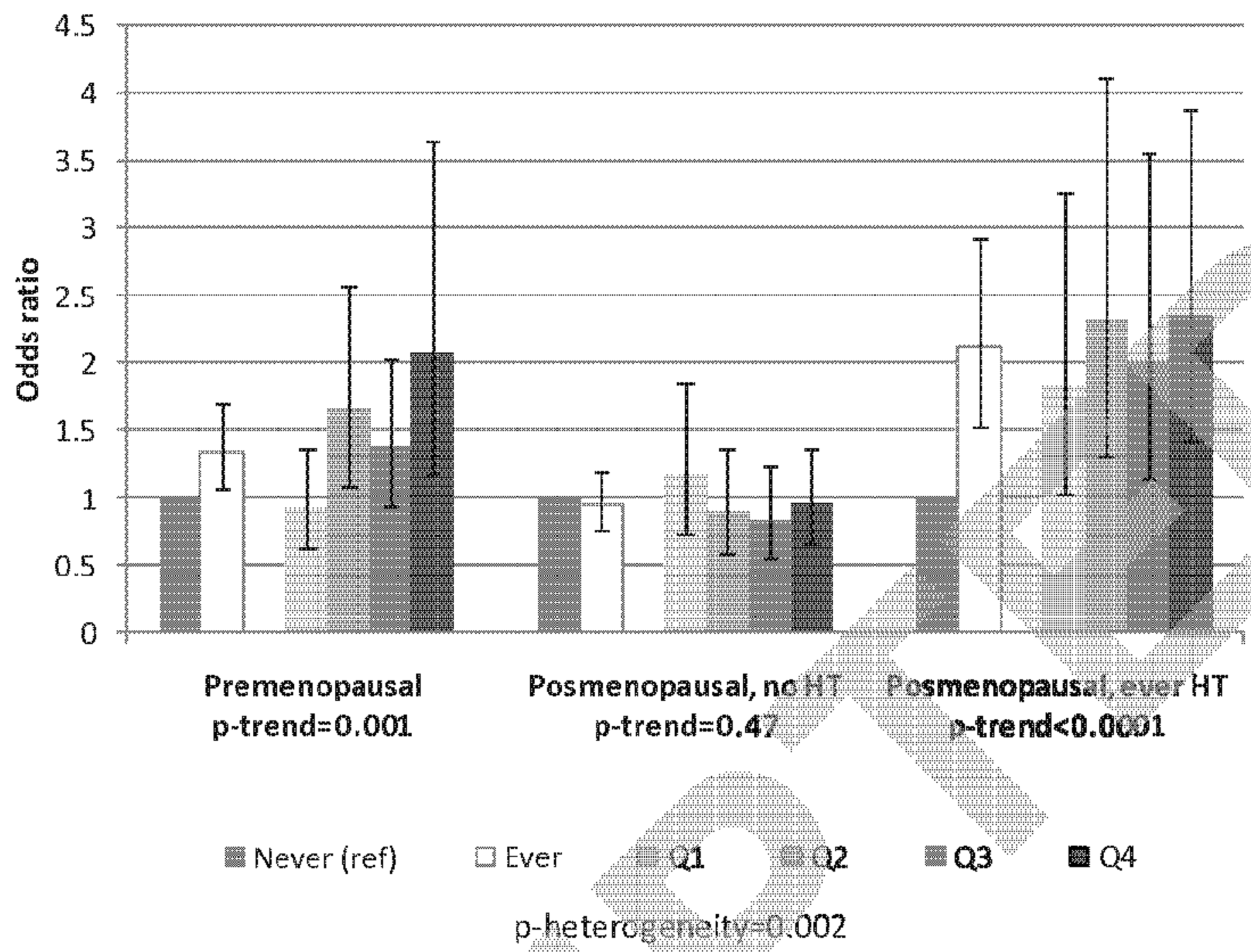
[a] Adjusted for reference age (continuous), study center, study phase (3, 4, 5), parity (nulliparous, 1, 2, ≥2), breast feeding (never, <4, 4-9, 10-19, >19 months), OC use (never, <23, 23-49, 50-96, >96 months), HT use (premenopausal, postmenopausal never used, postmenopausal used HT), IUD (never, ever), endometriosis or painful periods, personal history of breast cancer, Jewish ethnicity, tubal ligation, and BMI (<22.2, 22.2-24.8, 24.9-28.6, >28.6).

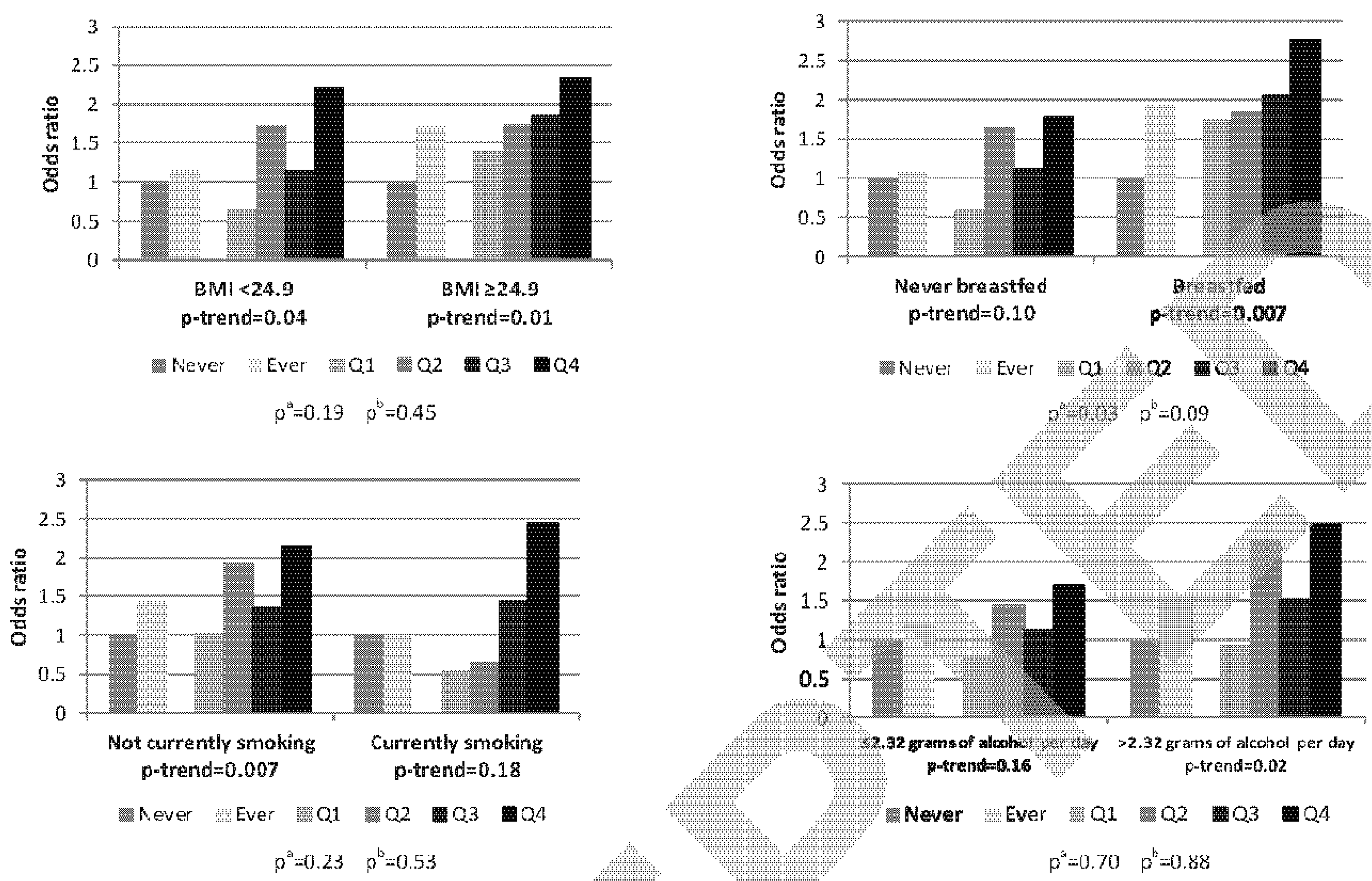
Table 5. Associations between genital talc use (never/ever and quartiles of talc-years) and ovarian cancer by menopausal status and postmenopausal hormone therapy among restricted histologic types.

Genital talc use	Premenopausal			Postmenopausal, never used HT			Postmenopausal, ever used HT		
	Controls N (%)	Cases[a] N (%)	Adjusted[b] OR (95% CI)	Controls N (%)	Cases[a] N (%)	Adjusted[b] OR (95% CI)	Controls N (%)	Cases[a] N (%)	Adjusted[b] OR (95% CI)
Never	735 (79)	531 (72)	1.00 (referent)	507 (69)	378 (69)	1.00 (referent)	309 (72)	152 (53)	1.00 (referent)
Ever	197 (21)	211 (28)	1.42 (1.12, 1.81)	230 (31)	173 (31)	1.00 (0.78, 1.28)	122 (28)	133 (47)	2.32 (1.64, 3.27)
No genital use	735 (79)	531 (72)	1.00 (referent)	507 (69)	378 (69)	1.00 (referent)	309 (72)	152 (54)	1.00 (referent)
≤1	70 (8)	47 (6)	0.90 (0.60, 1.37)	40 (6)	36 (7)	1.32 (0.80, 2.17)	28 (7)	28 (10)	2.02 (1.10, 3.70)
>1-5	44 (5)	52 (7)	1.66 (1.06, 2.60)	52 (7)	32 (6)	0.81 (0.50, 1.32)	28 (7)	29 (10)	2.56 (1.40, 4.67)
>5-24	59 (6)	68 (9)	1.54 (1.04, 2.28)	61 (8)	41 (8)	0.86 (0.55, 1.33)	26 (6)	30 (11)	2.18 (1.19, 4.00)
>24	21 (2)	41 (6)	2.33 (1.32, 4.12)	70 (10)	58 (10)	1.00 (0.88, 1.49)	36 (8)	43 (15)	2.57 (1.51, 4.36)
p-trend			0.0006			0.88			0.001

[a] Postmenopausal cases are restricted to serous and endometrioid invasive, premenopausal cases additionally include serous and mucinous borderline cases.
[b] Adjusted for reference age (continuous), study center, study phase (3, 4, 5), parity (nulliparous, 1, 2, ≥2), breast feeding (never, <4, 4-9, 10-19, >19 months), OC use (never, <23, 23-49, 50-96, >96 months), IUD (never, ever), endometriosis or painful periods, personal history of breast cancer, Jewish ethnicity, tubal ligation, and BMI (<22.2, 22.2-24.8, 24.9-28.6, >28.6).


Controls
Cases
Percent ever used talc on genitals
40
35
30
25
20
15
10
5
0
<30
30-39
<30
30-39
40-49
30-39
40-49
50-59
40-49
50-59
60-69
50-59
60-69
70+
60-69
70+
≥1971
1961-1970
1951-1960
1941-1950
1931-1940
≤1930
Reference age and year of birth


4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Odds ratio
Premenopausal
p-trend=0.001
Posmenopausal, no HT
p-trend=0.47
Posmenopausal, ever HT
p-trend<0.0001
Never (ref)
Ever
Q1
Q2
Q3
Q4
p-heterogeneity=0.002


Odds ratio
BMI <24.9
p-trend=0.04
BMI ≥24.9
p-trend=0.01
Never Ever Q1 Q2 Q3 Q4
p^a=0.19 p^b=0.45
Never breastfed
p-trend=0.10
Breastfed
p-trend=0.007
Never Ever Q1 Q2 Q3 Q4
p^a=0.03 p^b=0.09
Not currently smoking
p-trend=0.007
Currently smoking
p-trend=0.18
Never Ever Q1 Q2 Q3 Q4
p^a=0.23 p^b=0.53
≤2.32 grams of alcohol per day
p-trend=0.16
>2.32 grams of alcohol per day
p-trend=0.02
Never Ever Q1 Q2 Q3 Q4
p^a=0.70 p^b=0.88

Talc Use, Variants of the *GSTM1*, *GSTT1*, and *NAT2* Genes, and Risk of Epithelial Ovarian Cancer

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Abstract

Epidemiologic evidence suggests a possible association between genital use of talcum powder and risk of epithelial ovarian cancer; however, the biological basis for this association is not clear. We analyzed interactions between talc use and genes in detoxification pathways [*glutathione S-transferase M1* (*GSTM1*), *glutathione S-transferase T1* (*GSTT1*), and *N-acetyltransferase 2* (*NAT2*)] to assess whether the talc/ovarian cancer association is modified by variants of genes potentially involved in the response to talc. Our analysis included 1,175 cases and 1,202 controls from a New England-based case-control study and 210 cases and 600 controls from the prospective Nurses' Health Study. We genotyped participants for the *GSTM1* and *GSTT1* gene deletions and three *NAT2* polymorphisms. We used logistic regression to analyze the main effect of talc use, genotype, and gene-talc interactions in each population and pooled the estimates using a random-effects model. Regular talc use was associated with increased ovarian cancer risk in the combined study population (RR, 1.36; 95% CI, 1.14-1.63; $P_{trend} < 0.001$). Independent of talc, the genes examined were not clearly associated with risk. However, the talc/ovarian cancer association varied by *GSTT1* genotype and combined *GSTM1*/*GSTT1* genotype. In the pooled analysis, the association with talc was stronger among women with the *GSTT1*-null genotype ($P_{interaction}$ = 0.03), particularly in combination with the *GSTM1*-present genotype ($P_{interaction}$ = 0.03). There was no clear evidence of an interaction with *GSTM1* alone or *NAT2*. These results suggest that women with certain genetic variants may have a higher risk of ovarian cancer associated with genital talc use. Additional research is needed on these interactions and the underlying biological mechanisms. (Cancer Epidemiol Biomarkers Prev 2008;17(9):2436–44)

Introduction

Genital use of talcum powder has been extensively investigated as a potential risk factor for ovarian cancer. A meta-analysis of 16 previous studies reported an approximately 30% increase in risk of total epithelial ovarian cancer with regular genital exposure to talc (1), and several studies have suggested a stronger association with the serous or serous invasive histologic subtype (2-6). Although the epidemiologic evidence supports a modest association between genital talc use and ovarian cancer risk, the association remains controversial due to the lack of a clear dose-response with increasing frequency or duration of talc use, the possibility of confounding or other biases, and the uncertain biological mechanism.

No prior studies have assessed gene-talc interactions in ovarian cancer risk possibly because little is known about which genes may be involved in the biological response to talc. However, variants of the *glutathione S-transferase M1* (*GSTM1*) and *N-acetyltransferase 2* (*NAT2*) genes appear to modify the association between exposure to asbestos, a known carcinogen that is chemically similar to talc, and risk of malignant mesothelioma (7-10). Talc and asbestos are found together in nature, and before 1976, talcum powder was commonly contaminated with asbestos (9). Although this contamination may have contributed to the risk of ovarian cancer associated with talc use, there is also evidence that talc itself may contribute to carcinogenesis independent of any contamination with asbestos in the past. Talc can induce granulomas and other inflammatory responses *in vivo* (9), and a recent study found that exposing human ovarian stromal and epithelial cells to talc resulted in increased cell proliferation and neoplastic transformation of cells (11). Talc also appears to increase cellular production of reactive oxygen species (11). Interestingly, serous ovarian cancers morphologically resemble peritoneal malignant mesotheliomas (12), suggesting a possible rationale for the stronger association between talc and risk of serous or serous invasive cancers observed in some studies.

Based on similarities between talc and asbestos and the evidence for gene-asbestos interactions in malignant mesothelioma, we examined whether the association between genital talc exposure and ovarian cancer risk is modified by variants of the *NAT2* and *GSTM1* genes as

Received 5/1/08; revised 6/23/08; accepted 6/30/08.
Grant support: National Cancer Institute, NIH grants P50 CA105009, P01 CA87969, and R01 CA054419 and training grants T32 CA009001 and R25 CA098566.
Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).
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doi:10.1158/1055-9965.EPI-08-0399

well as the related *glutathione S-transferase T1* (*GSTT1*) gene. The *GSTM1* and *GSTT1* genes produce enzymes involved in the metabolism of carcinogens and reactive oxygen species (13). These genes are homozygously deleted in approximately 50% (*GSTM1*) and 20% (*GSTT1*) of Caucasians, resulting in complete loss of enzymatic activity (14, 15). The NAT2 enzyme catalyzes the deactivation of xenobiotics via *N*-acetylation but can also activate certain substrates via *O*-acetylation (16). Individuals with two *NAT2* slow acetylator alleles, approximately 60% of individuals in Caucasian populations, have decreased rates of *N*- and *O*-acetylation (17-20). We hypothesized that the association between talc use and ovarian cancer risk would be stronger among individuals with the *GSTM1*-null, *GSTT1*-null, and *NAT2* slow acetylator genotypes due to decreased metabolism of free radicals and other products of the biological response to talc. We examined these gene-talc interactions, as well as the main effect of talc use and each genotype, in two study populations with a total of 1,385 ovarian cancer cases.

Materials and Methods

New England Case-Control Study. The New England Case-Control Study (NECC) consists of 1,231 epithelial ovarian cancer cases and 1,244 controls from Massachusetts and New Hampshire. Participants were enrolled in the study in two phases: from May 1992 to March 1997 (phase I; 563 cases and 523 controls) or from July 1998 to July 2003 (phase 2; 668 cases and 721 controls). Participants completed a detailed questionnaire on potential risk factors for ovarian cancer and covariates of interest during an in-person interview with a trained interviewer. To avoid capturing changes related to disease status, interviewers asked participants about exposures that occurred at least 1 year before the date of diagnosis for cases or the interview date for controls. The institutional review boards of Brigham and Women's Hospital and Dartmouth Medical School approved both phases of the study, and all participants provided written informed consent.

During the two study phases, NECC researchers identified 2,347 incident cases of ovarian cancer through hospital tumor boards and state cancer registries; 1,845 (79%) of these cases were eligible and 71% of the eligible cases were enrolled in the study. Study investigators identified potential controls using random-digit dialing, drivers' license records, and Massachusetts' town resident lists. Controls were frequency matched to cases by age and state of residence. Of the potentially eligible controls contacted by investigators during phase 1, 68% were eligible and agreed to participate. During phase 2, 197 potential controls declined to be contacted by returning a postcard to "opt out" of the study; of the remaining potentially eligible controls who were contacted, 67% were eligible and enrolled in the study. The eligibility criteria and the reasons for nonenrollment of eligible cases are described elsewhere (21).

Over 95% of study participants provided a blood specimen at study enrollment. NECC researchers separated the heparinized blood samples into plasma, RBC, and buffy coat (WBC) components, extracted DNA from the buffy coat using Qiagen DNA extraction, and stored the extracted DNA in freezers at a temperature of -80°C.

Nurses' Health Study. In 1976, 121,701 female registered nurses between ages 30 and 55 years responded to a mailed questionnaire about known and suspected risk factors for disease, leading to the establishment of the Nurses' Health Study (NHS). Study participants completed follow-up questionnaires every 2 years, providing information on new diagnoses of disease and updated information on risk factors. Participation in the study has remained high throughout follow-up; between 1976 and 2004, the percentage of follow-up information obtained (questionnaire responses plus deaths) was 95.3%. The corresponding follow-up percentages for women who provided a WBC or cheek cell specimen were 98% and 99%, respectively. The Institutional Review Board of Brigham and Women's Hospital approved both the NHS and this analysis, and all participants provided implied consent by completing and returning the baseline questionnaire.

In 1989 and 1990, 32,826 participants submitted a blood sample for use in genetic and other biomarker analyses. Details of the blood collection are described elsewhere (22). Between 2001 and 2004, 33,040 women without a blood specimen provided a buccal cell specimen. We used a mouthwash protocol to collect the buccal cell samples, based on evidence that this method provides slightly higher DNA yield and quality, compared with collection using a cytobrush (23). We extracted DNA from each specimen within 1 week of receipt using Qiagen DNA extraction, and stored the DNA at -80°C.

NHS Nested Case-Control Study. We collected information on new diagnoses of ovarian cancer on each questionnaire and also obtained information on deaths due to ovarian cancer through family members, the National Death Index, and the U.S. Postal Service. We confirmed each diagnosis using methods described previously (24). For this analysis, we included all cases with a DNA specimen available from before diagnosis (incident cases) as well as cases who submitted a DNA specimen within 4 years after diagnosis (prevalent cases). We included the prevalent cases in the analysis due to the similarity of characteristics of these cases and the incident cases and also because the interval of 4 years between diagnosis and DNA collection was less than the average survival time of 65.7 months for the incident cases. All cases were diagnosed before June 1, 2004 and had no history of a prior cancer, other than nonmelanoma skin cancer.

We randomly selected three controls per case from the study participants who gave a buccal cell or blood specimen, who had not had a bilateral oophorectomy before the date of diagnosis of the matched case, and who had no history of cancer, other than nonmelanoma skin cancer, as of the cycle of diagnosis of the case. We excluded 30 controls from the analysis due to unavailability of genotyping data (n = 28) or because the participant was later diagnosed with ovarian cancer and was included in the analysis as a case (n = 2). Cases and controls were matched on month and year of birth, DNA type, and menopausal status at diagnosis. For the

blood collection, cases and controls were additionally matched on menopausal status and postmenopausal hormone (PMH) use status at blood draw, month/year and time of day of blood draw, and fasting status at blood draw, because these control selections were also used for analyses of plasma hormones and other biomarkers (25).

Exposure Assessment. The phase 1 and 2 NECC questionnaires included multiple questions about regular use of talcum, baby, or deodorizing powder as an adult. Specific questions asked about type of use (as a dusting powder to the genital area, sanitary napkins, underwear, or nongenital areas), frequency of use, age at first use, number of years used, and brand of powder used. The 1982 NHS questionnaire requested information on whether the participant had ever commonly applied talcum, baby, or deodorizing powder to the perineal area (no, less than once a week, 1-6 times a week, or daily) or to sanitary napkins (yes/no). For this analysis, we defined regular genital talc use as application of powder to the genital/perineal region at least once a week. We also created a categorical variable for frequency of talc use using the categories from the NHS questionnaire.

Genotyping Methods. Genotyping was done at the Dana-Farber/Harvard Cancer Center High Throughput Genotyping Core (for the *NAT2* polymorphisms and NHS *GSTM1* and *GSTT1* gene deletions) and the Molecular Epidemiology Research Laboratory at the Harvard School of Public Health (for the NECC *GSTM1* and *GSTT1* gene deletions). All samples were genotyped for three single nucleotide polymorphisms that identify the *NAT2*5*, *NAT2*6*, and *NAT2*7* alleles. These alleles account for over 99% of slow acetylator alleles in Caucasian populations (16, 26). The *NAT2* I114T (rs1801280), R197Q (rs1799930), and G286E (rs1799931) polymorphisms were genotyped using the 5′-nuclease assay (Taqman) on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) in 384-well format. Individuals with two slow acetylator alleles were classified as *NAT2* slow acetylators, whereas individuals with zero or one slow acetylator allele were classified as rapid acetylators.

The NECC samples were genotyped for the *GSTM1* and *GSTT1* gene deletions using multiplex PCR, and the PCR products were resolved on a 1.5% agarose gel. The NHS samples were genotyped for the two gene deletions using Taqman real-time PCR in 384-well format. For both multiplex and real-time PCR assays, individuals were considered to have the *GSTM1*- or *GSTT1*-null genotype if no PCR product was present for the respective gene; all other individuals were classified as *GSTM1*- or *GSTT1*-present.

All DNA samples were whole genome amplified before genotyping. Laboratory personnel blinded to the case-control status of the samples did all genotyping, and each plate included blinded replicate samples for quality-control purposes. The replicate samples were 100% concordant for all genotypes, except the NECC *GSTM1* and *GSTT1* gene deletions, which were 98% and 95% concordant, respectively.

Statistical Analysis. We used a χ^2 test to examine whether the *NAT2* polymorphisms were in Hardy-Weinberg equilibrium in each population and also to examine the distribution of each genotype by case-control status. We conducted all analyses separately in the NHS and NECC populations using consistent exposure and covariate definitions and, after testing for heterogeneity in the results, pooled the estimates using a random-effects model (27). We used conditional (NHS) and unconditional (NECC and NHS) logistic regression to model the multivariable-adjusted odds ratio [as an estimate of the relative risk (RR)] and 95% confidence interval (95% CI) for the main effect of genital talc use, the main effect of each gene, and each combined gene-talc variable. We tested for a linear trend with increasing frequency of talc use by using a continuous variable weighted by the midpoint of each frequency category and calculated the *P* value for trend using the Wald test. To assess effect modification by genotype, we used unconditional logistic regression to model the association between talc use and ovarian cancer risk within each genotype stratum and calculated the *P* value for interaction using the χ^2 test for the difference between the log likelihood for models with and without interaction terms between regular genital talc use and genotype. In addition to the analyses of total ovarian cancer, we examined associations with the serous invasive histologic subtype based on evidence from prior studies that risk of this subtype may be more strongly associated with talc use.

We adjusted all analyses for the matching factors, duration of oral contraceptive use, parity, tubal ligation, body mass index (BMI), and duration of PMH use. Women with missing data for the continuous covariates were assigned the median value of the covariate for their study population. In the NHS, where covariate data are available from multiple questionnaire cycles, we used the data from two cycles (2-4 years) before the cycle of diagnosis for each case and their matched controls for consistency with the timeframe of the NECC covariate data. We examined additional covariates as potential confounders, including physical activity, smoking history, menopausal status, age at menopause, breastfeeding duration, and family history of ovarian or breast cancer but did not include them in the final model because they did not substantially change our estimates. We did all analyses using SAS version 9.1 (SAS Institute).

Results

Our study population included 1,175 cases and 1,202 frequency-matched controls from the NECC and 210 cases and 600 matched controls from the NHS for a total of 1,385 ovarian cancer cases and 1,802 controls. Of the NHS cases, 49 were prevalent and 161 were incident with respect to the time of DNA collection. Characteristics of the NHS prevalent and incident cases were generally similar, although a higher percentage of the prevalent cancers were endometrioid (20% versus 9%) and a lower percentage were invasive (76% versus 86%). In the NECC, 618 cases had serous histology (53%), 450 were serous invasive (38%), 153 were mucinous (13%), 172 were endometrioid (15%), and 232 had other/undifferentiated histology (20%). In the NHS, 111 cases were serous (53%), 93 were serous

invasive (44%), 23 were mucinous (11%), 25 were endometrioid (12%), and 51 had other/poorly differentiated histology (24%).

Over 96% of the NECC participants and 98% of the NHS participants were of self-reported European ancestry. In analyses restricted to these participants, the results were similar to those for the entire study population; we therefore included all participants in our analyses to maximize our sample size. The distributions of ovarian cancer risk factors were similar in the NECC and NHS populations, although on average the NHS participants were older, had higher parity, and were more likely to have used PMH in part due to differences in the NECC and NHS age distributions (Table 1). Within each study population, the cases and controls differed with respect to the known risk factors for ovarian cancer. In addition, in the NECC, the cases had higher mean BMI than the controls, and a larger percentage of the cases reported a history of genital talc use. The NHS prevalent and incident cases had similar BMI, tubal ligation history, duration of PMH use, duration of lactation, and genital talc use history; however, the prevalent cases were, on average, slightly younger (60 versus 62 years), were less likely to be postmenopausal (71% versus 87%), and had lower parity (2.7 versus 3.1 children), later age at menarche (13.1 versus 12.5 years), and a longer mean duration of oral contraceptive use (60 versus 41 months; results not shown).

In the NECC, women with a history of regular genital talc use were older, had higher mean BMI, were less likely to have ever used oral contraceptives, were more likely to be postmenopausal, and were more likely to have used PMH (Table 2). Among parous women in the NHS, the mean age at first birth was lower for regular talc users. In addition, NHS participants who regularly used talc were less likely to have a history of smoking or tubal ligation. There was no difference in the genotype frequencies by genital talc use history in either study population.

All P values for the tests for heterogeneity comparing the NECC and NHS results were >0.05. Talc use was associated with increased risk of ovarian cancer in both study populations, although the 95% CIs were wide in the NHS due to the limited sample size (Table 3). In the pooled analysis, the RR for the association with regular genital talc use was 1.36 (95% CI, 1.14-1.63) for total ovarian cancer and 1.60 (95% CI, 1.26-2.02) for the serous invasive subtype. In addition, there were highly significant trends between increasing frequency of talc use and risk of both total and serous invasive ovarian cancer in the NECC (P_{trend} = 0.002 for total and P_{trend} < 0.001 for serous invasive ovarian cancer) and pooled analyses (P_{trend} < 0.001 for both total and serous invasive ovarian cancer). Regular genital talc use was not significantly associated with risk of the endometrioid (RR, 1.41; 95% CI, 0.97-2.05) or mucinous (RR, 1.28; 95% CI, 0.85-1.92) histologic subtypes in the pooled analysis. In the NECC, use of talcum powder on nongenital body areas was unassociated with ovarian cancer risk (multivariable-adjusted RR, also adjusted for genital talc use = 0.91; 95% CI, 0.73-1.12).

Among the controls in each population, the genotype frequencies for the *NAT2* polymorphisms were in Hardy-Weinberg equilibrium and the distributions of

Table 1. Characteristics of ovarian cancer cases and controls in the NECC and the NHS

Characteristic	NECC			NHS*		
	Cases	Controls	P †	Cases	Controls	P †
N	1,175	1,202		210	600	
Mean (SD)						
Age (y)‡	51 (13)	51 (13)	0.37	62 (8)	62 (8)	0.93
Parity among parous women	2.5 (1.3)	2.8 (1.5)	<0.001	3.0 (1.3)	3.4 (1.5)	0.004
Duration oral contraceptive use (mo)§	52 (54)	61 (55)	0.006	46 (42)	53 (49)	0.22
BMI (kg/m^2)	26.3 (6.3)	25.7 (5.5)	0.02	25.7 (5.0)	25.7 (4.5)	0.94
Duration PMH use (mo)§	78 (86)	74 (71)	0.64	96 (84)	85 (68)	0.18
Duration of lactation (mo)∥	5.0 (10.0)	7.3 (13.2)	<0.001	6.0 (10.3)	7.2 (9.7)	0.17
Percent of study population						
Parous	68	81	<0.001	89	93	0.05
Ever user of oral contraceptives	48	60	<0.001	42	45	0.57
History of tubal ligation	14	18	0.007	14	21	0.02
Ever user of PMH	17	20	0.14	71	63	0.02
Family history of ovarian cancer	5.1	2.8	0.004	9.1	3.7	0.002
Any history of genital talc use	29	24	0.003	40	39	0.79
Regular genital talc use (once a week or more)	27	20	<0.001	29	24	0.15
Daily genital talc use	16	12	0.006	18	13	0.08
Genotype frequencies, %						
GSTM1 null	51	53	0.42	48	52	0.36
GSTT1 null	21	22	0.85	19	21	0.45
NAT2 slow acetylator¶	63	64	0.74	59	67	0.05

*In the NHS, duration of lactation was collected in 1986, family history of ovarian cancer was first collected in 1992, and history of genital talc use was collected in 1982; for variables collected on multiple questionnaires, the value from two cycles (2-4 y) before the date of diagnosis for each case was used for the case and their matched controls.
†P values calculated using proc t test (continuous variables) or a χ^2 test (binary variables).
‡Cases and controls in each study population were matched (NHS) or frequency-matched (NECC) on age.
§Duration of oral contraceptive use and PMH use among ever users.
∥Total duration among parous women.
¶*NAT2* acetylation genotype based on analysis of three single nucleotide polymorphisms, I114T, R197Q, and G286E.

Table 2. Characteristics of participants in the NECC and the NHS by history of regular genital talc use (at least once a week)

Characteristic	NECC			NHS*		
	No regular talc use	Regular talc use	P †	No regular talc use	Regular talc use	P †
Mean (SD)						
Age (y)	50 (13)	53 (12)	<0.001	61 (8)	62 (8)	0.64
Parity among parous women	2.7 (1.4)	2.7 (1.4)	0.64	3.2 (1.4)	3.3 (1.5)	0.42
Age at first birth among parous women	25.0 (5.1)	24.6 (4.9)	0.22	25.0 (3.5)	24.4 (3.0)	0.03
Duration oral contraceptive use (mo)‡	58 (55)	54 (54)	0.24	53 (49)	43 (42)	0.08
BMI (kg/m^2)	25.7 (5.7)	27.0 (6.4)	<0.001	25.6 (4.6)	26.2 (4.8)	0.13
Duration PMH use (mo)‡	75 (74)	78 (86)	0.68	90 (74)	83 (70)	0.38
Duration of lactation (mo)§	6.4 (12.1)	5.8 (11.5)	0.32	7.2 (10.2)	6.3 (9.6)	0.34
Physical activity (h/wk)	2.8 (5.0)	2.4 (3.8)	0.06	3.0 (2.3)	3.1 (2.4)	0.61
Percent of study population						
Parous	74	75	0.75	93	92	0.81
Ever user of oral contraceptives	55	50	0.03	44	44	0.96
History of tubal ligation	16	16	0.97	22	13	0.008
Postmenopause	45	54	<0.001	81	81	0.86
Ever user of PMH	17	26	<0.001	66	64	0.73
Ever smoker	53	55	0.35	57	47	0.02
Family history of ovarian cancer	3.9	4.1	0.82	4.5	6.4	0.31
Genotype frequencies, %						
GSTM1 null	52	52	0.72	51	49	0.66
GSTT1 null	21	22	0.59	22	17	0.15
NAT2 slow acetylator‖	63	66	0.23	65	63	0.58

*In the NHS, duration of lactation was collected in 1986, family history of ovarian cancer was first collected in 1992, and history of genital talc use was collected in 1982; for variables collected on multiple questionnaires, the value from two cycles (2-4 y) before the date of diagnosis for each case was used for the case and their matched controls.
†P values calculated using proc ttest (continuous variables) or a χ^2 test (binary variables).
‡Duration of oral contraceptive use and PMH use among ever users.
§Total duration among parous women.
‖*NAT2* acetylation genotype based on analysis of three single nucleotide polymorphisms, I114T, R197Q, and G286E.

the *GSTM1*-null, *GSTT1*-null, and *NAT2* slow acetylator genotypes were consistent with previous reports of Caucasian populations (19, 28, 29). Comparing the prevalent and incident cases in the NHS, a nonsignificantly higher percentage of the prevalent cases were *NAT2* slow acetylators (67% versus 56%), but the *GSTM1* and *GSTT1* genotype distributions did not differ for the prevalent and incident cases (results not shown).

None of the genotypes examined were associated with ovarian cancer risk in the NECC or pooled analyses (Table 4). In the NHS, individuals with the *NAT2* slow acetylator genotype had a significant 35% decrease in ovarian cancer risk (RR, 0.65; 95% CI, 0.45-0.95). The combined *GSTM1*-null/*NAT2* slow acetylator and *GSTT1*-null/*NAT2* slow acetylator genotypes were also inversely associated with risk in the NHS (RR, 0.57; 95% CI, 0.33-0.98 and RR, 0.51; 95% CI, 0.26-0.99, respectively) when compared with the *GSTM1*- or *GSTT1*-present, *NAT2* rapid acetylator genotype. However, these associations were no longer statistically significant when pooled with the NECC estimates.

In analyses stratified by genotype, the association between regular genital talc use and risk of total ovarian cancer was stronger among women with the *GSTT1*-null and combined *GSTM1*-present/*GSTT1*-null genotypes (Table 5). In the pooled analysis, the RR for the association with regular genital talc use was 2.1 (95% CI, 1.4-3.2) for women with the *GSTT1*-null genotype ($P_{interaction} = 0.03$) and 2.8 (95% CI, 1.6-5.0) for women with the *GSTM1*-present/*GSTT1*-null genotype ($P_{interaction} = 0.03$). The association with the serous invasive subtype was also stronger within these genotype strata, although the P values for interaction were not statistically significant. The pooled RR was 2.4 (95% CI, 1.4-4.0) for the *GSTT1*-null stratum and 4.8 (95% CI, 2.1-11) for the combined *GSTM1*-present/*GSTT1*-null stratum. The results were consistent in both study populations (results not shown), although the P values for interaction were statistically significant only in the pooled analysis. There was also evidence of a stronger association between regular talc use and risk of serous invasive cancer among women with the *GSTM1*-present genotype, but this interaction was not statistically significant.

We additionally analyzed the association between combined gene-talc variables, compared with a common reference group (wild-type genotype and no talc use), and risk of total and serous invasive ovarian cancer. The results of these analyses were similar to the stratified results presented in Table 5 and are therefore included only as a supplementary table. We also examined interactions between regular genital talc use and combined *GSTM1/NAT2* and *GSTT1/NAT2* genotype (results not shown). The *GSTT1*-null/*NAT2* slow acetylator genotype seemed to increase the risk of total and serous invasive ovarian cancer associated with talc use. However, these analyses were based on small numbers, especially for certain combinations of the genotype and talc variables, and none of the P values for interaction were significant.

In analyses restricted to the NHS incident cases or the NHS cases and controls with a blood specimen, the results were similar to those for the total NHS study population (results not shown).

Table 3. RR (95% CI) for the association between genital talc use and ovarian cancer risk in the NECC and the NHS

	NECC*			NHS*			Pooled †
	Cases (%)	Controls (%)	RR (95% CI)	Cases (%)	Controls (%)	RR (95% CI)	RR (95% CI)
Total epithelial ovarian cancer:							
N ‡	1,175	1,202		210	600		
Regular genital talc use (once a week or more)							
No	859 (73.2)	957 (79.7)	1.00 (reference)	138 (70.8)	414 (76.0)	1.00 (reference)	1.00 (reference)
Yes	314 (26.8)	244 (20.3)	1.40 (1.15-1.70)	57 (29.2)	131 (24.0)	1.24 (0.83-1.83)	1.36 (1.14-1.63)
Frequency of genital talc use							
Never	832 (70.9)	916 (76.3)	1.00 (reference)	120 (61.5)	352 (64.6)	1.00 (reference)	1.00 (reference)
Less than once a week	27 (2.3)	41 (3.4)	0.72 (0.43-1.19)	18 (9.2)	62 (11.4)	0.98 (0.54-1.79)	0.82 (0.55-1.20)
1-6 times a week	123 (10.5)	96 (8.0)	1.33 (1.00-1.79)	22 (11.3)	61 (11.2)	1.01 (0.57-1.79)	1.26 (0.97-1.63)
Daily	191 (16.3)	148 (12.3)	1.41 (1.10-1.79)	35 (18.0)	70 (12.8)	1.44 (0.88-2.37)	1.41 (1.14-1.76)
P_{trend} §			0.002			0.18	<0.001
Serous invasive ovarian cancer:							
N ‡	450	1,202		93	263		
Regular genital talc use (once a week or more)							
No	310 (69.0)	957 (79.7)	1.00 (reference)	60 (68.2)	177 (73.8)	1.00 (reference)	1.00 (reference)
Yes	139 (31.0)	244 (20.3)	1.62 (1.26-2.09)	28 (31.8)	63 (26.3)	1.48 (0.82-2.68)	1.60 (1.26-2.02)
Frequency of genital talc use							
Never	299 (66.6)	916 (76.3)	1.00 (reference)	54 (61.4)	151 (62.9)	1.00 (reference)	1.00 (reference)
Less than once a week	11 (2.4)	41 (3.4)	0.65 (0.32-1.33)	6 (6.8)	26 (10.8)	0.79 (0.29-2.11)	0.70 (0.39-1.24)
1-6 times a week	56 (12.5)	96 (8.0)	1.56 (1.08-2.26)	12 (13.6)	25 (10.4)	1.64 (0.71-3.79)	1.58 (1.12-2.21)
Daily	83 (18.5)	148 (12.3)	1.61 (1.18-2.20)	16 (18.2)	38 (15.8)	1.31 (0.65-2.76)	1.56 (1.17-2.08)
P_{trend} §			<0.001			0.29	<0.001

*Unconditional (NECC) and conditional (NHS) logistic regression adjusted for age, study center (NECC only), duration of oral contraceptive use (months), parity (continuous), tubal ligation, BMI (kg/m^2, continuous), and duration of PMH use (months).
†P values for tests for heterogeneity comparing the NECC and NHS results were all >0.38.
‡Frequencies do not add up to total N due to missing data on talc use.
§Weighted by the midpoint of each category of genital talc use frequency and calculated using the Wald test.

Discussion

These results provide additional support for a main effect of genital talc exposure on risk of epithelial ovarian cancer. The presence of a significant trend between frequency of talc use and risk of total and serous invasive ovarian cancer in the NECC and pooled analyses further strengthens the evidence for an association, as most previous studies have not observed a dose response with increasing frequency or duration of talc use (1, 5). The results of our gene-environment analyses suggest that genes in detoxification pathways may be involved in the biological response to talc and that the association between genital talc use and risk of ovarian cancer may vary by genotype. In particular, women with the *GSTT1*-null genotype and the combined *GSTM1*-present/*GSTT1*-null genotype had a stronger association between talc use and ovarian cancer risk. The evidence for these interactions was consistent in two independent study populations, and the P values for interaction were statistically significant in a pooled analysis of the two populations. However, the direction of the interaction with combined *GSTM1/GSTT1* genotype was unexpected based on the known function of these genes.

Although prior analyses of the talc/ovarian cancer association in the NHS and the NECC have been published, our study includes an additional 612 NECC cases and 679 NECC controls and 8 additional years of follow-up in the NHS (3, 4). In the previous analysis of the NECC, Cramer et al. observed a significant positive association between talc use and risk of both total and serous invasive ovarian cancer. In addition, there was a significant trend with lifetime number of talc applications, after excluding applications during nonovulatory intervals (P_{trend} = 0.02), but no trend with duration or frequency of talc use (3). In the only prospective study of this association, Gertig et al. reported a significant association between talc use and risk of the serous invasive subtype in the NHS but no association with risk of total ovarian cancer (4). Our findings are consistent with the previous reports for these study populations, although our analysis differs from the prior studies in that we defined our primary exposure variable as genital use of talc at least once per week based on the assumption that habitual talc use is more likely to be recalled accurately and more likely to be associated with ovarian cancer risk. Our findings are also consistent with meta-analyses of this association (1, 30).

The controversy regarding the existence of an association between talc and ovarian cancer has stemmed in part from the lack of a clear mechanism for the association. Although talc and asbestos are chemically similar, their biological effects may differ, because talc does not appear to be a lung carcinogen (31). In addition, it is unclear whether talc applied to the perineum can reach the ovaries, although some studies have shown that inert particles can travel through the female genital tract to the fallopian tubes and ovaries (32, 33), and others have found talc particles in ovarian tissue (34-37). Recent studies have suggested additional potential mechanisms for an association between talc and ovarian cancer. Talc particles can induce an inflammatory response *in vivo*, which may be important in ovarian cancer risk (38). Normal ovarian cells treated with talc are more likely to undergo cell proliferation and neoplastic transformation, and cellular generation of reactive oxygen species increases with increasing exposure to talc (11). Recent studies by Cramer et al. also support the possibility of an

immune-mediated mechanism for an association between talc and ovarian cancer and suggest that exposure of the lower genital tract to talc may be sufficient to cause changes, such as production of heat shock proteins, accumulation of talc in pelvic lymph nodes, or decreased levels of anti-MUC1 antibodies, which could increase ovarian cancer risk (39-41).

Although no prior studies have examined gene-talc interactions, the indication of a possible immune-related mechanism between talc and ovarian carcinogenesis and the evidence for gene-asbestos interactions suggest that genes involved in detoxification and inflammatory pathways could be important in the response to talc. Previous studies have indicated that *NAT2* and *GSTM1* genotype may modify the association between asbestos exposure and risk of malignant mesothelioma; however, not all studies have been consistent (7, 8, 42, 43), and for *NAT2*, the direction of the interaction differed in studies conducted in Finnish and Italian populations (7, 8, 42, 44). This suggests that interactions with these genes may be complex and might depend on additional factors, such as the presence of other gene variants, the type of asbestos, or the level of asbestos exposure (8).

The *GSTM1* and *GSTT1* genes produce enzymes that metabolize products of oxidative stress and catalyze the detoxification of carcinogens and other xenobiotics (45). The *GSTM1* deletion and, to a lesser extent, the *GSTT1* deletion may increase the risk of certain cancers; however, our study and previous analyses do not support a direct association between *GSTM1* or *GSTT1* gene deletion and risk of ovarian cancer (13, 17, 28). Although there is some overlap in GST substrate specificity, there are also differences in the substrates metabolized by the GSTM1 and GSTT1 enzymes, which could help to explain the opposite direction of the interactions we observed between talc use and *GSTM1* and *GSTT1* genotype (13, 17, 45). In studies of pleural malignant mesothelioma, the *GSTM1*-null genotype was associated with increased risk (7, 8, 42, 43) while the *GSTT1*-null genotype was unassociated with risk of malignant mesothelioma (8, 42, 43) but was associated with a significant decrease in risk of asbestosis in one study (46), providing support that some functions of the GSTM1 and GSTT1 enzymes may differ. The direction of the associations between *GSTM1* and *GSTT1* deletions and risk of asbestos-related disease was opposite to the direction of the interactions with talc observed in our study; this could potentially be due to differences in the chemical structures of talc and asbestos or differences in the byproducts produced during the biological response to talc and asbestos. The NAT2 enzyme catalyzes the transfer of an acetyl group to its substrates, including carcinogens such as heterocyclic and aromatic amines, which can result in either activation or deactivation of these substances (17, 20). Approximately 60% of Caucasians have two *NAT2* slow acetylator alleles and consequently have decreased rates of acetylation, which can either increase or decrease the risk of certain cancers depending on the substrate and the cancer site (17, 20). To our knowledge, no previous studies have examined the association between *NAT2* slow acetylator genotype and ovarian cancer risk. We did not observe strong evidence of a main effect of *NAT2* genotype or an interaction between *NAT2* genotype and talc exposure.

Table 4. RR (95% CI) for the association between *GSTM1*, *GSTT1*, and *NAT2* genotype and epithelial ovarian cancer risk in the NECC and the NHS

	NECC*			NHS*			Pooled †
	Cases (%)	Controls (%)	RR (95% CI)	Cases (%)	Controls (%)	RR (95% CI)	RR (95% CI)
N ‡	1,175	1,202		210	600		
GSTM1 genotype							
Present	573 (49.1)	567 (47.4)	1.00 (reference)	102 (52.3)	268 (48.5)	1.00 (reference)	1.00 (reference)
Null	594 (50.9)	628 (52.6)	0.93 (0.79-1.10)	93 (47.7)	285 (51.5)	0.83 (0.58-1.17)	0.91 (0.78-1.06)
GSTT1 genotype							
Present	919 (78.8)	938 (78.5)	1.00 (reference)	157 (81.3)	439 (78.8)	1.00 (reference)	1.00 (reference)
Null	247 (21.2)	257 (21.5)	0.98 (0.80-1.21)	36 (18.7)	118 (21.2)	0.87 (0.57-1.33)	0.96 (0.80-1.16)
NAT2 genotype							
Rapid/intermediate acetylator	387 (36.8)	405 (36.1)	1.00 (reference)	77 (41.0)	182 (33.0)	1.00 (reference)	1.00 (reference)
Slow acetylator	665 (63.2)	717 (63.9)	0.97 (0.81-1.15)	111 (59.0)	369 (67.0)	0.65 (0.45-0.95)	0.82 (0.57-1.20)
Combined *GSTM1/GSTT1* genotype							
Both present	445 (38.2)	430 (36.0)	1.00 (reference)	81 (44.3)	206 (39.2)	1.00 (reference)	1.00 (reference)
M1 null, *T1* present	474 (40.7)	508 (42.5)	0.91 (0.76-1.10)	68 (37.2)	208 (39.5)	0.82 (0.54-1.22)	0.89 (0.75-1.06)
M1 present, *T1* null	128 (11.0)	137 (11.5)	0.94 (0.71-1.24)	17 (9.3)	49 (9.3)	0.98 (0.52-1.84)	0.94 (0.73-1.22)
Both null	119 (10.2)	120 (10.0)	0.94 (0.70-1.26)	17 (9.3)	63 (12.0)	0.65 (0.34-1.24)	0.88 (0.67-1.15)
Combined *GSTM1/NAT2* genotype							
GSTM1 present, *NAT2* rapid	195 (18.6)	188 (16.9)	1.00 (reference)	37 (21.0)	85 (16.4)	1.00 (reference)	1.00 (reference)
GSTM1 null, *NAT2* rapid	189 (18.1)	214 (19.2)	0.82 (0.61-1.09)	35 (19.9)	91 (17.5)	0.96 (0.53-1.74)	0.84 (0.65-1.09)
GSTM1 present, *NAT2* slow	315 (30.1)	343 (30.7)	0.86 (0.66-1.11)	56 (31.8)	161 (31.0)	0.87 (0.51-1.50)	0.86 (0.68-1.09)
GSTM1 null, *NAT2* slow	347 (33.2)	371 (33.2)	0.88 (0.68-1.14)	48 (27.3)	183 (35.2)	0.57 (0.33-0.98)	0.76 (0.50-1.14)
Combined *GSTT1/NAT2* genotype							
GSTT1 present, *NAT2* rapid	296 (28.3)	312 (28.0)	1.00 (reference)	52 (29.7)	144 (27.5)	1.00 (reference)	1.00 (reference)
GSTT1 null, *NAT2* rapid	88 (8.4)	90 (8.1)	1.03 (0.73-1.45)	17 (9.7)	30 (5.7)	1.55 (0.76-3.16)	1.11 (0.81-1.52)
GSTT1 present, *NAT2* slow	519 (49.7)	562 (50.4)	0.97 (0.79-1.19)	90 (51.4)	270 (51.6)	0.87 (0.57-1.33)	0.95 (0.79-1.14)
GSTT1 null, *NAT2* slow	142 (13.6)	152 (13.6)	0.98 (0.74-1.31)	16 (9.1)	79 (15.1)	0.51 (0.26-0.99)	0.76 (0.40-1.43)

*Unconditional (NECC) and conditional (NHS) logistic regression adjusted for age, study center (NECC only), duration of oral contraceptive use (months), parity (continuous), tubal ligation, BMI (kg/m^2, continuous), and duration of PMH use (months).
†*P* values for tests for heterogeneity comparing the NECC and NHS results were all >0.06.
‡Frequencies do not add up to total *N* due to missing genotype data.

Table 5. Pooled RR (95% CI) for the association between regular talc use and ovarian cancer risk, stratified by genotype, in the NECC and the NHS

	All cancers		Serous invasive cancers		Cases and controls in pooled analysis					
					All cases		Serous invasive		Controls	
	Regular talc use		Regular talc use		Regular talc		Regular talc		Regular talc	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Gene/stratum										
GSTM1 genotype										
Present (+)	1.0 (reference)	1.6 (1.2-2.0)	1.0 (reference)	2.0 (1.4-2.8)	480	189	173	90	646	165
Null (-)	1.0 (reference)	1.3 (1.0-1.6)	1.0 (reference)	1.4 (1.0-1.9)	498	179	190	76	690	198
$P_{interaction}$*	0.13		0.08							
GSTT1 genotype										
Present (+)	1.0 (reference)	1.2 (1.0-1.5)	1.0 (reference)	1.5 (1.2-2.0)	785	278	288	129	1,035	301
Null (-)	1.0 (reference)	2.1 (1.4-3.2)	1.0 (reference)	2.4 (1.4-4.0)	194	87	71	38	300	67
$P_{interaction}$*	0.03		0.18							
NAT2 genotype										
Rapid/intermediate acetylator	1.0 (reference)	1.5 (1.1-2.0)	1.0 (reference)	1.9 (1.2-2.8)	330	128	123	57	459	113
Slow acetylator	1.0 (reference)	1.4 (1.1-1.8)	1.0 (reference)	1.6 (1.2-2.1)	552	217	204	96	819	233
$P_{interaction}$*	0.60		0.58							
GSTM1/GSTT1 genotype†										
GSTM1+, GSTT1+	1.0 (reference)	1.4 (1.0-1.8)	1.0 (reference)	1.7 (1.2-2.5)	378	142	136	68	479	140
GSTM1-, GSTT1+	1.0 (reference)	1.2 (0.9-1.5)	1.0 (reference)	1.4 (0.9-1.9)	400	135	151	60	541	154
GSTM1+, GSTT1-	1.0 (reference)	2.8 (1.6-5.0)	1.0 (reference)	4.8 (2.1-11)	98	47	34	22	158	24
GSTM1-, GSTT1-	1.0 (reference)	1.6 (0.9-2.9)	1.0 (reference)	1.4 (0.6-3.1)	94	40	36	16	138	41
$P_{interaction}$*	0.03		0.09							

NOTE: NECC: unconditional logistic regression adjusted for age, study center, duration of oral contraceptive use (months), parity (continuous), tubal ligation, BMI (kg/m^2, continuous), and duration of PMH use (months); NHS: unconditional logistic regression adjusted for age (months), menopausal status at diagnosis (post, pre/dubious), DNA source, duration of oral contraceptive use (months), parity (continuous), tubal ligation, BMI (kg/m^2, continuous), and duration of PMH use (months). P values for tests for heterogeneity comparing the NECC and NHS results were all >0.36.
*P values for interaction based on likelihood ratio test comparing unconditional logistic regression models with and without gene-talc interaction terms.
†NHS analysis adjusted for age, menopausal status at diagnosis, and DNA source only to improve stability of estimates.

The novelty of this analysis and the assessment of gene-talc interactions in two independent study populations, one with a large number of cases and the other with prospective data on talc use and ovarian cancer incidence, are strengths of this study. However, although the pooled analysis included a large number of cases and controls, our power was still insufficient to detect interactions with certain combinations of genes and for specific histologic subtypes. In addition, although both study populations had extensive covariate data, the use of common exposure and covariate definitions resulted in the loss of some detail particularly for the NECC. Information on talc use was only collected in 1982 in the NHS, so it is possible that some participants were misclassified with respect to their talc use history. However, the number of participants who began using talc after 1982, when the participants were between ages 36 and 61 years, is most likely small. Although we do not have data on age at initiation of talc use in the NHS, in the NECC, approximately 95% of controls with a history of regular genital talc use reported first using talc before age 35 years. Recall or selection bias may have affected the results of the NECC analyses due to the retrospective study design. However, the consistency of the NECC and NHS results suggests that biases related to study design were not a major problem, because, with the exception of the DNA for a subset of the cases, the NHS data were collected prospectively. In addition, the exposure definition of genital talc use at least once a week may have decreased the influence of recall bias in this analysis, because habitual talc use is likely to be recalled more accurately than sporadic use.

In summary, our findings suggest that variants of the *GSTM1* and *GSTT1* genes may modify the association between genital talc use and risk of total and serous invasive ovarian cancer. However, additional research is needed to confirm these findings and to explore potential mechanisms for these interactions particularly for the stronger talc/ovarian cancer association among women with the *GSTM1*-present/*GSTT1*-null genotype. If confirmed, these findings would strengthen the evidence for the carcinogenicity of talc to the ovarian epithelium.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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We thank Hardeep Ranu, Pati Soule, Shireen Sarraf, and Jason Wong for laboratory technical assistance and the participants of the NECC and the NHS for their dedication to these studies and contribution to this research.

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Cancer Epidemiology, Biomarkers & Prevention

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Talc Use, Variants of the *GSTM1, GSTT1*, and *NAT2* Genes, and Risk of Epithelial Ovarian Cancer

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Cancer Epidemiol Biomarkers Prev 2008;17:2436-2444.

Published in final edited form as:
Obstet Gynecol. 2011 May ; 117(5): 1042–1050. doi:10.1097/AOG.0b013e318212fcb7.

Assessing Ovarian Cancer Risk When Considering Elective Oophorectomy at the Time of Hysterectomy

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Abstract

Objective—To develop a risk-factor score that may provide additional guidance to women and their physicians regarding elective bilateral salpingo-oophorectomy at the time of hysterectomy.

Methods—From a case–control study conducted from 1992 to 2008 in women residing in eastern Massachusetts or New Hampshire, we selected 1,098 women with invasive ovarian cancer (case group) and 1,363 for the control group who were older than 40 years and had neither hysterectomy nor a personal or family history of breast or ovarian cancer. Using logistic regression, we identified key risk factors and built a risk score. The score was separately assessed in 126 women in the case group and 156 in the control group with excluded prior hysterectomy to determine whether women who developed ovarian cancer could have been distinguished.

Results—Summing eight conditions found to be associated with ovarian cancer (Jewish ethnicity, less than 1 year of oral contraceptive use, nulliparity, no breastfeeding, no tubal ligation, painful periods or endometriosis, polycystic ovary syndrome or obesity, talc use), we created a five-level score. Assigning average risk to those with a score of 2, the odds ratios varied from 0.56 (95% confidence interval [CI] 0.42–0.74) for a score of 0–1 to 3.30 (95% CI 2.50–4.35) for a score of 5 or greater (*P* trend <.001). The risk score was higher for women who developed ovarian cancer after hysterectomy than those who did not (*P*=.01). Lifetime risks for ovarian cancer for a woman at age 40 years are changed from 1.2% with a 0–1 score to 6.6% with a score of 5 or higher.

Conclusion—We developed a risk-assessment tool that can quantify women's risk for ovarian cancer and should be validated in other data sets.

In the 1990s, there were approximately 600,000 hysterectomies performed in the United States annually and 55% of these also involved bilateral salpin-go-oophorectomy,[1] many done solely to reduce the risk for ovarian cancer. It has been suggested that elective bilateral salpingo-oophorectomy be considered for women older than 40 years,[2–4] whereas surveys in the United Kingdom revealed that 85–90% of physicians recommended bilateral salpingo-oophorectomy for postmenopausal women coming to hysterectomy.[5,6] However, Parker et al,[7] citing evidence that postmenopausal ovaries secrete androgens important to health, performed a risk–benefit analysis and concluded that ovarian conservation benefits long-term survival for women at "average risk" for ovarian cancer undergoing hysterectomy for benign disease. A subsequent study using observational data from the Nurses' Health Study

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Financial Disclosure: The authors did not report any potential conflicts of interest.

on all and various causes of mortality for hysterectomized women with and without oophorectomy supported their conclusion.[8]

In addressing the value of bilateral salpingo-oophorectomy, Parker et al distinguished average-risk women from those with known *BRCA1* or *BRCA2* mutations or a strong family history of breast and ovarian cancer. In the latter group, bilateral salpingo-oophorectomy may truly be beneficial in reducing risk for both breast and ovarian cancer.[9] Genetic or familial risk factors or both, however, account for a small proportion of ovarian cancer. Consequently, it is important to assess ovarian cancer risk among women who lack the genetic or familial profile. In this article, we describe a risk-factor score that may be of value in further categorizing risk for ovarian cancer in women without a personal or family history of cancer to provide additional guidance to women and their physicians regarding elective bilateral salpingo-oophorectomy at the time of hysterectomy.

Materials and Methods

Data used in this study come from three enrollment phases of a case–control study of ovarian cancer in New England. The earlier two phases have been described previously.[10] Briefly, we used statewide cancer registries and hospital tumor boards to identify ovarian cancer cases diagnosed in eastern Massachusetts and the entire state of New Hampshire from May 1992 to March 1997 and August 1998 to April 2003. We enrolled 1,306 women in the case group of whom 1,231 had been diagnosed with epithelial ovarian cancers. Women for the control group for the first phase of the study were identified by random-digit dialing supplemented with residents' lists for older control-group participants. Approximately 10% of households randomly dialed contained an eligible control and of these, 421 (72%) agreed to participate. All women for the control group for the second phase were identified through town resident lists (town books) in Massachusetts and drivers' license registries in New Hampshire. Of the 2,102 potential control-group participants identified through town books in both phases, 635 were ineligible, 644 declined participation, and 823 were enrolled. In total, 1,244 women were enrolled in the control group.

In the third enrollment phase, between October 2003 and November 2008, we identified 1,610 women residing in eastern Massachusetts or New Hampshire who had a diagnosis of incident ovarian cancer. Of these, 372 could not be contacted because they had died, moved, or had no telephone; did not speak English; had a nonovarian primary tumor after review; or lived outside the study area. Physicians declined permission to contact 128, and 213 declined or were too ill to participate. The remaining 897 women were enrolled; of these, 845 had epithelial ovarian tumors, including tumors of borderline malignancy.

Similar to the second phase of the study, control-group participants were identified through town books in Massachusetts and drivers' license lists in New Hampshire. Age matching was accomplished by sampling control-group participants based on the age distribution of women in the case group in the previous phases of the study with adjustment as current case-group participants were enrolled. Of the 2,523 potential control-group participants identified, 850 were ineligible because they had died, moved, had no telephone, did not speak English, had no ovaries, or were seriously ill and 816 potential control-group participants declined participation either by phone or by "opt out" postcard. A total of 857 control-group participants were enrolled.

In all phases, after written informed consent, demographic information, reproductive and medical history, and habits were assessed by in-person interview. All of the questions were framed with respect to a reference date defined as 1 year before the diagnosis date for

women in the case group and the date of interview for those in the control group. Histologic type, grade, and stage of disease were abstracted from case pathology reports. This study was approved by Brigham and Women's Hospital and Dartmouth Medical Center's institutional review boards.

We used two approaches to identify women who may be at greater risk for ovarian cancer after hysterectomy and more likely to benefit from elective bilateral salpingo-oophorectomy. In the first approach, we constructed a risk-factor score that would be relevant to decision-making for "average-risk" women coming to hysterectomy. For this analysis, we excluded all women who had prior hysterectomy (n=368). We also excluded women who would be deemed to be at above-average risk because of a personal history of breast cancer or a family history of ovarian cancer at any age or breast cancer diagnosed before age 50 years (n=532). We excluded women younger than 40 years because they would unlikely be offered bilateral salpingo-oophorectomy without an indication (n=615). We also restricted the analysis to women who had an invasive ovarian cancer, whose survival is substantially worse compared with those with borderline tumors. The final sample included 1,098 women in the case group (including 17 primary peritoneal cases) and 1,363 in the control group. In the second approach, also after excluding borderline cases and women with a personal or family history of breast or ovarian cancer, we examined women in the case (n=126, including one primary peritoneal case) and control groups (n=156) who had previous hysterectomy to determine whether risk profiles or reasons for the surgery could have distinguished women who subsequently developed ovarian cancer.

In both approaches, unconditional logistic regression models were used to identify significant risk factors distinguishing women in the case group from those in the control group. Continuous variables were categorized based on quartiles of the control distributions. Associations are presented as odds ratios, 95% confidence intervals, and Wald test *P* values. We used Wald tests from logistic regression to test for trends in ordinal categorical exposures by creating ordinal variables in which the median value or midpoint of each category was assigned to all participants within that category. To evaluate whether associations between risk factors and ovarian cancer varied by study phase, we conducted stratified analyses and likelihood ratio tests comparing models with both main effects and interaction terms with models with main effects only. Because of the small amount of missing data in this study, participants with missing exposures were dropped from analyses. Combinations of factors were examined to identify the best cumulative index of experiences associated with ovarian cancer risk. In all models, we adjusted for study phase and the matching variables age (continuous) and study site (Massachusetts, New Hampshire).

We translated the relative risks obtained from the model into absolute risks by multiplying them by cumulative risks for ovarian cancer occurrence with age 85 years as an end point. Cumulative risks were calculated from 2003 2007 age-specific incidence rates for ovarian cancer provided through Surveillance, Epidemiology, and End Results (SEER) of the National Cancer Institute.[11] These rates are based on all women as the denominator including women with an oophorectomy, whereas we wish cumulative risk to apply only to women with intact ovaries. From a study that examined the effect of hysterectomy and oophorectomy on genital cancer rates,[12] we adjusted age-specific incidence rates upward based on estimates of the prevalence of oophorectomy by dividing each age-specific incidence rate by one minus the prevalence of oophorectomy in that age group. Cumulative incidence was calculated by summing the adjusted age-specific incidence rates times the duration of the age-specific incidence intervals as described in Rothman and Greenland.[13]

Results

Table 1 shows the distribution of women in the case and control groups by study details and well-established or potential risk factors for ovarian cancer. The majority of women enrolled in the case group were white, which limited our ability to include race as a risk factor. We observed highly significant increases in risk associated with lack of oral contraceptive use, nulliparity, never having breastfed, no tubal ligation, painful periods or endometriosis, polycystic ovarian syndrome or obesity (body mass index [calculated as weight (kg)/[height (m)]^2] greater than 30), and long-term genital talc use. An increasing number of estimated ovulatory cycles not interrupted by pregnancies, breastfeeding, or oral contraceptive use was also strongly associated with increased risk. Having a Jewish ethnic background was associated with increased risk but of borderline significance (P=.08). There was no significant association with age at menarche or menopause, fertility hormones, or menopausal hormone use (except for progesterone-only regimens, which were used by few participants in this study). We observed no significant interactions between risk factors and study phase.

The final entry in Table 1 shows the results of a simple score created to summarize risk by number of ovarian cancer risk factors. Conditions included in this score are Jewish ethnicity, more than 1 year of oral contraceptive use, nulliparity, no breastfeeding, no tubal ligation, painful periods or endometriosis, polycystic ovarian syndrome or obesity, and long-term genital talc use. There was a significant trend of increasing risk with increasing number of conditions (Ptrend <.001). Compared with women with two conditions, women with zero to one condition had a 40% reduction in risk, whereas women with three, four, and five or more conditions had 60%, twofold, and threefold increases in risk, respectively. We examined this score by histologic subtype and stage of invasive epithelial ovarian cancer and observed significant trends of increasing risk for all subtypes and early- and late-stage disease (Table 2).

Table 3 shows the results of the analysis of ovarian cancer in women in the case and control groups who had prior hysterectomy. There were significant trends for risk of ovarian cancer to be lower with an older age at hysterectomy and greater with a longer interval since performance of the hysterectomy. The most common reasons for hysterectomy (by the woman's self-report) were heavy bleeding, leiomyomas, or both, which were diagnosed in 61.9% of women in the case groups and 57.0% of those in the control group. Compared with this group, there was a lower likelihood for developing ovarian cancer if the reported diagnosis was prolapse (P=.06). Risk of ovarian cancer among hysterectomized women increased monotonically with a higher risk-factor score (Ptrend=.01). The average risk-factor score was 3.4 for all women in the case group compared with 3.0 for all women in the control group (P=.009) and 3.4 for women in the case group compared with 2.6 for those in the control group (P=.01) for women who underwent hysterectomy after age 45 years. Women with ovarian cancer who had prior hysterectomy had a higher frequency of serous histologic types (67%) and lower frequency of endometrioid and clear cell types (22%) compared with nonhysterectomized women in the case group, in which the respective frequencies were 52% and 36% (P<.001) (data not shown).

Table 4 translates the risk-factor score from Table 1 into absolute risks for the occurrence of ovarian cancer during the remaining years of life from a particular starting age beginning at age 40 years until age 85 years as an end point. Assuming that the category of two risk factors best represents risk in the general population (and therefore the referent category), we multiplied the cumulative risks by 0.6, 1.6, 2.1, and 3.3 for the score categories 0–1, 3, 4, and 5 or more, respectively. As illustrated in Table 4, a woman who is 40 years old and has zero to one risk factors would have an absolute risk of developing ovarian cancer by age 85

years of 1.2% (95% CI 0.8–1.4%), whereas a woman with five or more risk factors would have a risk of 6.6% (95% CI 5.0–8.6%).

Discussion

Current American College of Obstetricians and Gynecologist guidelines[14] recommend that family history, menopausal status, and pelvic disease that might predispose to reoperation be considered in whether bilateral salpingo-oophorectomy should be offered to women coming to hysterectomy. The guidelines state that "Strong consideration should be made for retaining normal ovaries in premenopausal woman who are not at increased genetic risk of ovarian cancer." Bilateral salpingo-oophorectomy should be offered to women with known or suspected *BRCA1* or *BRCA2* mutations after completion of childbearing. For postmenopausal women (with normal ovaries), the guidelines state: "Given the risk of ovarian cancer in postmenopausal women, ovarian removal at the time of hysterectomy should be considered for these women." Nulligravidity and family history of ovarian cancer are mentioned as increasing risk for ovarian cancer; and pregnancy, tubal ligation, and use of oral contraceptive are mentioned as decreasing risk. However, no concrete rules are offered on how these characteristics might be used to weigh risk in an individual woman.

In this article, we derive a simple score to help physicians and women weigh individual risk for ovarian cancer. We first excluded those women who would already be viewed at high risk such as those with a personal history of breast cancer or family history (of a mother or sister) with breast cancer (before age 50 years) or ovarian cancer at any age. To make the model most relevant to women considering oophorectomy at the time of hysterectomy, we then excluded women younger than 40 years, who may be inappropriate candidates for elective oophorectomy without known ovarian pathology, as well as women in the case and control groups who had prior hysterectomy. We identified those risk factors to be considered: parity, oral contraceptive use, breastfeeding, tubal ligation, painful periods or endometriosis, obesity or polycystic ovarian syndrome, and talc use. These risk factors are concordant with published epidemiologic data related to reproductive factors,[15–23] use of talc,[17–19] tubal ligation,[20,24–27] endometriosis,[28] and polycystic ovarian syndrome or obesity.[29,30] It is also known that approximately 2% of Jewish women carry one of three founder mutations of *BRCA1* or *BRCA2*. Approximately 40% of Jewish women who present with ovarian cancer will carry a founder mutation.[31] Even after removing those with a family history of breast or ovarian cancer, women with Jewish ethnic backgrounds remain at approximately a 30% increased in risk for ovarian cancer.

Creating simple dichotomies from these factors and summing them allowed a five-level risk score to be constructed, which correlated directly with increasing relative risks for ovarian cancer. Combining various risk factors to create a risk score for ovarian cancer has been performed in studies that have looked at the estimated number of ovulatory cycles, which also directly correlates with ovarian cancer risk.[10,32] However, we did not include ovulatory cycles in our model because estimating them would require a calculator or paper and pencil. Thus, a simple linear combination of diverse risk factors, even those that do not logically fit into an ovulatory cycles score, adds cumulatively to increase ovarian cancer risk. We previously have discussed the potential basis for this phenomenon as indicating a common pathway for many ovarian cancer risk factors operating through their ability to affect immunity related to important cell surface glycoproteins, know as mucins, especially MUC1.[33]

We also performed an analysis on women who had previous hysterectomy. Most case–control studies of ovarian cancer allow women with hysterectomy to be included in the control group as long as they said their operation did not include oophorectomy. Nearly all

hysterectomized women who later developed ovarian cancer would be correct in their recollection that they did not have a bilateral salpingo-oophorectomy. However, there is a greater likelihood that those who did not develop ovarian cancer may have incorrectly stated their ovaries were left, leading to misclassification. We are uncertain whether this may partially explain the greater percentage of control-group participants who reported hysterectomy without bilateral salpingo-oophorectomy after age 46 years compared with women in the case group observed in this study. Because historical medical records could not be retrieved for participants, it was also necessary to rely on the woman's recollection of why the surgery was performed. Women who went on to develop ovarian cancer after hysterectomy were less likely to have had hysterectomy for prolapse (P=.06). Regarding our risk score, we again found a significant trend for a higher cumulative score to predict greater risk for ovarian cancer occurring after hysterectomy. Notably, the average score for women who had hysterectomy after age 45 years and subsequently developed ovarian cancer was 3.4 for women in the case group compared with 2.6 for those in the control group (P=.01). It may be particularly important to initiate a dialogue about ovarian cancer risk factors before hysterectomy after this age.

Potential weaknesses of this study derive from the fact that case–control data were used to create our scoring system. Biases may occur in case–control studies that can affect risk estimates, including recall bias leading to misclassification of exposure. In addition, selection biases may occur in that exposures for women with rapidly fatal disease who could not be interviewed may be underrepresented. Nevertheless, the risk factors we observed agree with published data, some of which come from cohort studies in which these biases are less likely to occur and our scoring system was applied to both early- and late-stage disease (Table 2). Another limitation of case–control data is that it allows only relative, not absolute, risks to be calculated directly. To overcome this limitation, we multiplied the odds ratio for each score by estimated lifetime risks of ovarian cancer. The age-specific incidence rates used to calculate lifetime risks were first adjusted upward based on the prevalence of oophorectomy in the general population.

Our risk score does not provide a precise formula for when elective oophorectomy should be recommended because we did not perform a cost–benefit analysis taking into consideration the competing risks from long-term complications of bilateral salpingooophorectomy, including bone fracture and cardiovascular diseases. Based on the rarity of ovarian cancer relative to other conditions considered by Parker et al in their analysis of the Nurses' Health Data, it is possible that, even if all cases of ovarian cancer could be predicted and eliminated, overall benefits might not be shifted toward selective bilateral salpingo-oophorectomy. Nevertheless, we think it is important for physicians and their patients to weigh individual risk for ovarian cancer when considering elective oophorectomy and have a discussion about individual risk for ovarian cancer. Even if the woman at elevated risk elects to conserve ovaries, bilateral salpingectomy without oophorectomy might be considered. Emerging evidence suggests that many high-grade invasive ovarian cancers may have their origin in the fallopian tubes rather than ovaries,[34] prompting Canadian health officials in British Columbia to urge gynecologists to perform salpingectomy (without oophorectomy) on women coming for hysterectomy. Our risk score might enable selection of women who would be candidates for this surgical alternative to oophorectomy if women at higher risk do not elect to have oophorectomy. Although we believe our scoring system is an improvement over existing methods for assessing risk for ovarian cancer in women without a family history, it should be viewed as a prototype until it can be validated in other data sets, especially with prospectively collected data from women including more nonwhites who were underrepresented in our study.

Acknowledgments

Supported by the National Cancer Institute grants to D.W.C.: Ovarian Cancer SPORE P50 CA105009 and R01 CA54419.

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Table 1

Conditions and Exposures Associated With Invasive Ovarian Cancer

	No. of Women in the Case Group (n = 1,098)	No. of Women in the Control Group (n = 1,363)	OR (95% CI)*	P
Study				
Phase 1: 1992–1997	284 (25.9)	316 (23.2)		
Phase 2: 1998–2003	327 (29.8)	456 (33.5)		
Phase 3: 2003–2008	487 (44.4)	591 (43.4)		
Site				
Massachusetts	860 (78.3)	1,117 (82.0)		
New Hampshire	238 (21.7)	246 (18.0)		
Race				
White	1,056 (96.2)	1,335 (98.0)	1.00	
African American	15 (1.4)	11 (0.8)	1.79 (0.82–3.93)	.15
Hispanic	9 (0.8)	12 (0.9)	1.02 (0.42–2.43)	.97
Asian	14 (1.3)	3 (0.2)	6.28 (1.80–21.9)	.004†
Other	4 (0.4)	2 (0.2)	2.52 (0.46–13.8)	.29
Jewish ethnicity				
No	1,017 (92.6)	1,283 (94.1)	1.00	
Yes	81 (7.4)	80 (5.9)	1.34 (0.97–1.85)	.08
Oral contraceptive use				
1 y or more	436 (39.7)	726 (53.3)	1.00	
Less than 1 y or no use	662 (60.3)	637 (46.7)	1.81 (1.52–2.15)	<.001
Parity				
Parous	794 (72.3)	1,162 (85.2)	1.00	
Nulliparous	304 (27.7)	201 (14.8)	2.34 (1.91–2.87)	<.001
Breastfeeding				
Any	353 (32.2)	690 (50.6)	1.00	
None	745 (67.8)	673 (49.4)	2.18 (1.84–2.57)	<.001
Tubal ligation				
Yes	142 (12.9)	294 (21.6)	1.00	
No	956 (87.1)	1,069 (78.4)	1.87 (1.50–2.33)	<.001
Pain with periods or endometriosis				
No	642 (58.5)	925 (67.9)	1.00	
Yes	456 (41.5)	438 (32.1)	1.53 (1.30–1.81)	<.001
PCOS or obesity (BMI more than 30 kg/m^2)				
No	785 (71.5)	1,039 (76.2)	1.00	
Yes	313 (28.5)	324 (23.8)	1.27 (1.06–1.52)	.01
Long-term genital talc use (10 y or more)				
No	932 (84.9)	1,211 (88.8)	1.00	
Yes	166 (15.1)	152 (11.2)	1.42 (1.12–1.81)	.004
Ovulatory cycles				
Quartile 1	149 (14.5)	317 (25.0)	1.00	

	No. of Women in the Case Group (n = 1,098)	No. of Women in the Control Group (n = 1,363)	OR (95% CI)*	P
Quartile 2	218 (21.2)	316 (24.9)	1.51 (1.16–1.97)	.002
Quartile 3	300 (29.1)	317 (25.0)	2.14 (1.65–2.77)	<.001
Quartile 4	363 (35.2)	319 (25.1)	2.63 (2.02–3.43)	<.001
Early menarche (younger than 12 y)				
Younger than 12	237 (21.7)	283 (20.8)	1.03 (0.85–1.25)	.77
12–15	815 (74.5)	1,006 (74.0)	1.00	
Older than 15	42 (3.8)	71 (5.2)	0.73 (0.49–1.08)	.11
Age at natural menopause (y)				
Younger than 49	243 (33.2)	283 (33.0)	1.00	
49–51	228 (31.1)	272 (31.7)	0.99 (0.77–1.27)	.93
Older than 51	262 (35.7)	303 (35.3)	1.03 (0.81–1.32)	.80
Postmenopausal hormone use				
None	839 (76.8)	983 (72.6)	1.00	
Estrogen only	54 (5.0)	77 (5.7)	0.77 (0.54–1.12)	.18
Estrogen and progesterone	174 (15.9)	245 (18.1)	0.83 (0.66–1.03)	.10
Progesterone only	4 (0.4)	17 (1.2)	0.28 (0.09–0.84)	.02
Oral contraceptives	3 (0.3)	8 (0.6)	0.46 (0.12–1.73)	.25
Other	18 (1.6)	25 (1.8)	0.82 (0.44–1.52)	.52
Fertility hormones				
No	1,014 (92.4)	1,255 (92.1)	1.00	
Yes	84 (7.6)	108 (7.9)	0.97 (0.72–1.31)	.84
Total number of risk factors‡				
0–1	98 (8.9)	311 (22.8)	0.56 (0.42–0.74)	<.001
2	201 (18.3)	361 (26.5)	1.00	
3	312 (28.4)	340 (24.9)	1.66 (1.31–2.09)	<.001
4	255 (23.2)	222 (16.3)	2.10 (1.64–2.70)	<.001
5 or more	232 (21.1)	129 (9.5)	3.30 (2.50–4.35)	<.001

OR, odds ratio; CI, confidence interval; PCOS, polycystic ovarian syndrome; BMI, body mass index.

Data are n (%) unless otherwise specified.

*Adjusted for study center, reference age, and study phase.

†The excess of Asian ovarian cancer cases simply may reflect limited ability to recruit Asian women for the control group.

‡Risk factors include Jewish ethnicity, less than 1 year of oral contraceptive use, nulliparity, no breastfeeding, no tubal ligation, painful periods or endometriosis, PCOS or BMI greater than 30 kg/m^2, and long-term talc use.

Table 2

Cumulative Index of Experiences Associated With Invasive Ovarian Cancer by Histologic Type and Stage

Total No. of Risk Factors*	Serous Invasive (n=566)†	Mucinous (n=62)†	Endometrioid (n=223)†	Clear Cell (n=175)†	Other or Undifferentiated (n=72)†	Early Stage (I–II) (n=462)†	Late Stage (III–IV) (n=634)†
0–1	0.56 (0.39–0.80)	0.61 (0.24–1.56)	0.66 (0.36–1.20)	0.34 (0.16–0.71)	0.88 (0.32–2.40)	0.52 (0.33–0.82)	0.58 (0.42–0.81)
2	1.00	1.00	1.00	1.00	1.00	1.00	1.00
3	1.39 (1.04–1.84)	1.62 (0.79–3.33)	2.38 (1.50–3.77)	1.43 (0.88–2.33)	3.56 (1.66–7.63)	2.11 (1.51–2.96)	1.43 (1.09–1.87)
4	1.65 (1.22–2.24)	1.46 (0.65–3.27)	3.02 (1.86–4.89)	2.92 (1.82–4.68)	2.95 (1.28–6.83)	3.28 (2.32–4.63)	1.55 (1.16–2.09)
5 or more	2.75 (1.98–3.82)	2.01 (0.86–4.75)	5.78 (3.54–9.42)	3.54 (2.11–5.93)	3.16 (1.25–7.99)	5.17 (3.58–7.47)	2.39 (1.73–3.30)
P trend	<.001	.01	<.001	<.001	<.001	<.001	<.001

Data are odds ratio (95% confidence interval) unless otherwise specified.

*Risk factors include Jewish ethnicity, less than 1 year of oral contraceptive pill use, nulliparity, no breastfeeding, no tubal ligation, painful periods or endometriosis, polycystic ovarian syndrome or body mass index greater than 30 kg/m^2, and long-term talc use.

†Adjusted for study center, reference age, and study phase.

Table 3

Hysterectomy Details and Cumulative Index of Experiences Among Women With Invasive Ovarian Cancer and Women in the Control Group Who Had Hysterectomy and Who Had No Personal History of Breast Cancer, Family History of Ovarian Cancer, or Early-Onset Breast

	No. of Women in the Case Group (n = 126)	No. of Women in the Control Group (n=156)	OR (95% CI)*	P
Age at hysterectomy (y)				
Younger than 35	35 (27.8)	36 (23.1)	1.00	
35–40	44 (34.9)	43 (27.6)	0.96 (0.50–1.82)	.89
41–46	30 (23.8)	39 (25.0)	0.77 (0.39–1.52)	.45
Older than 46	17 (13.5)	38 (24.4)	0.42 (0.20–0.90)	.02
*P*trend				.02
Time between hysterectomy and reference date (y)				
10 or less	27 (21.4)	42 (28.8)	1.00	
11 20	19 (15.1)	39 (25.0)	0.87 (0.39 1.92)	.72
21–30	45 (35.7)	43 (27.6)	1.84 (0.84–4.03)	.13
More than 30	35 (27.8)	29 (18.6)	2.17 (0.84–5.60)	.11
*P*trend				.04
Reason for hysterectomy				
Leiomyomas or heavy periods	78 (61.9)	89 (57.0)	1.00	
Endometriosis	10 (7.9)	13 (8.3)	0.92 (0.38–2.24)	.86
Prolapsed uterus	9 (7.1)	22 (14.1)	0.45 (0.19–1.05)	.06
Other	29 (23.0)	32 (20.5)	0.98 (0.54–1.77)	.94
Total number of risk factors†				
0–1	11 (8.7)	23 (14.7)	0.97 (0.39–2.38)	.94
2	21 (16.7)	41 (26.3)	1.00	
3	33 (26.2)	34 (21.8)	1.88 (0.92–3.86)	.08
4	33 (26.2)	35 (22.4)	1.83 (0.89–3.76)	.10
5 or more	28 (22.2)	23 (14.7)	2.45 (1.14–5.28)	.02
*P*trend				.01

OR, odds ratio; CI, confidence interval.

Data are n (%) unless otherwise specified.

* Adjusted for study center, reference age, and study phase.

† Risk factors include Jewish ethnicity, less than 1 year of oral contraceptive use, nulliparity, no breastfeeding, no tubal ligation, painful periods or endometriosis, polycystic ovarian syndrome or body mass index greater than 30 kg/m^2, and long-term talc use. The score was adjusted to estimate that which would have been observed before hysterectomy.

Table 4

Cumulative Risk of Developing Ovarian Cancer by Age 85 Years Using Oophorectomy-Adjusted Cumulative Incidence and the Relative Risks Associated With Each Level of the Risk-Factor Score

Total No. of Risk Factors	Probability of Developing Ovarian Cancer by Age 85 y Starting at Age								
	40	45	50	55	60	65	70	75	80
0–1	1.2 (0.8–1.4)	1.2 (0.8–1.4)	1.1 (0.8–1.3)	1.1 (0.7–1.3)	1.0 (0.6–1.1)	0.8 (0.6–1.0)	0.7 (0.4–0.8)	0.5 (0.3–0.6)	0.2 (0.2–0.3)
2*	2.0	2.0	1.9	1.8	1.6	1.4	1.1	0.8	0.4
3	3.2 (2.6–4.2)	3.2 (2.6–4.2)	3.0 (2.5–4.0)	2.9 (2.3–3.8)	2.6 (2.1–3.4)	2.2 (1.8–2.9)	1.8 (1.4–2.3)	1.3 (1.0–1.7)	0.6 (0.5–0.8)
4	4.2 (3.2–5.4)	4.2 (3.2–5.4)	4.0 (3.0–5.1)	3.8 (2.9–4.9)	3.4 (2.6–4.3)	2.9 (2.2–3.8)	2.3 (1.8–3.0)	1.7 (1.3–2.2)	0.8 (0.6–1.1)
5 or more	6.6 (5.0–8.6)	6.6 (5.0–8.6)	6.3 (4.8–8.2)	5.9 (4.5–7.7)	5.3 (4.0–6.9)	4.6 (3.5–6.0)	3.6 (2.8–4.7)	2.6 (2.0–3.4)	1.3 (1.0–1.7)

* Two risk factors was chosen as the referent category.

Data are cumulative risk (95% confidence interval).

Original Article

International Journal of Toxicology
1-9
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DOI: 10.1177/1091581815607068
ijt.sagepub.com
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Inhalation of Talc Induces Infiltration of Macrophages and Upregulation of Manganese Superoxide Dismutase in Rats

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Abstract

Talc is a mineral that is widely used in cosmetic products, antiseptics, paints, and rubber manufacturing. Although the toxicological effects of talc have been studied extensively, until now no detailed inhalation study of talc focusing on oxidative stress has been done. This repeated 4 weeks whole-body inhalation toxicity study of talc involved Sprague-Dawley rats. Male and female groups of rats were exposed to inhaled talc at 0, 5, 50, and 100 mg/m^3 for 6 hours daily, 5 days/week for 4 weeks. The objective was to identify the 4-week inhalation toxicity of talc and investigate antioxidant activity after exposure to talc. There were no treatment-related symptoms or mortality in rats treated with talc. Glucose (GLU) was decreased significantly in male rats exposed to 50 and 100 mg/m^3 of talc. Histopathological examination revealed infiltration of macrophages on the alveolar walls and spaces near the terminal and respiratory bronchioles. In male and female rats exposed to 100 mg/m^3 talc, expression of superoxide dismutase 2, a typical biological indicator of oxidative damage, was significantly increased. Thus, inhalation of talc induces macrophage aggregations and oxidative damage in the lung.

Keywords

talc, inhalation, SOD2, GPx1, macrophage

Introduction

Talc, also called talcum powder, is a mineral widely used in cosmetic products, antiseptics, ceramic industry, paints, confectionery food products, heat-resistant products, and rubber manufacturing.[1-3] The chemical formula of talc is $Mg_3Si_4O_{10}(OH)_2$ and it is a relatively pure substance although it can contain small quantities of aluminum, chromium, iron, manganese, and nickel.[4,5] Talc feels greasy because its hardness is low, and talc particles have usually thin plate-like shape, but the dimensions of each plate alter among diverse body of ore.[6]

Inhalation is the major pathway of human exposure of powdered toxic materials, and the lung is the first target organ. Adverse effects of inhaled toxicants include pulmonary disorders, lipid peroxidation, and DNA breakage.[7-10] Inhalation of a large quantity of talcum powder can cause short-term pulmonary talcosis.[1] One case report described that talc-induced pneumoconiosis was due to repeated abuse of talc-adulterated marijuana, and others reported that inhalation of talc caused lung diseases such as talc pneumoconiosis, bronchiolar obstruction and bronchiolitis, or even deaths.[1-3,11-19] Moreover, perineal talc use has been linked with ovarian cancer.[20-23]

Animal studies have shown that inhalation of talc can lead to lung injuries that include pulmonary inflammation, impaired phagocytosis, interstitial fibrosis, epithelial hyperplasia, and pulmonary edema.[24-27] Focal areas of papillary change in the surface epithelium of the ovary were described following intrabursal injection in rats.[28] Hemorrhage, edema, inflammation, proliferation, and fibrosis in lungs of rats exposed intrapleurally to talc slurry have been described.[29]

In contrast, no inhalation study has focused on oxidative stress. Oxidative stress induced by an inhaled toxicant could produce substantial quantities of superoxide anion, and the antioxidant enzyme superoxide dismutase (SOD) plays an important role in protection from the toxicity of the superoxide free radical.[30-32] Another important antioxidant enzyme in biological systems is glutathione peroxidase (GPx), which

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scavenges hydrogen peroxide and lipid peroxides.[33] Glutathione peroxidase is a selenium-containing enzyme that uses the sulfhydryl groups of reduced glutathione (GSH) as a hydrogen donor, catalyzing the formation of oxidized glutathione or glutathione disulfide.[34]

The present study was undertaken to identify the 4-week inhalation toxicity of talc and to investigate the expression of antioxidant enzymes in rat lung after inhalation exposure to talc. While many studies have shown the hazardous effects due to inhalation of talc in rats, no study has dealt with the oxidative effects in tissues of rats. In addition, hematological, biochemical, and histopathological examinations were done, and talc components were analyzed.

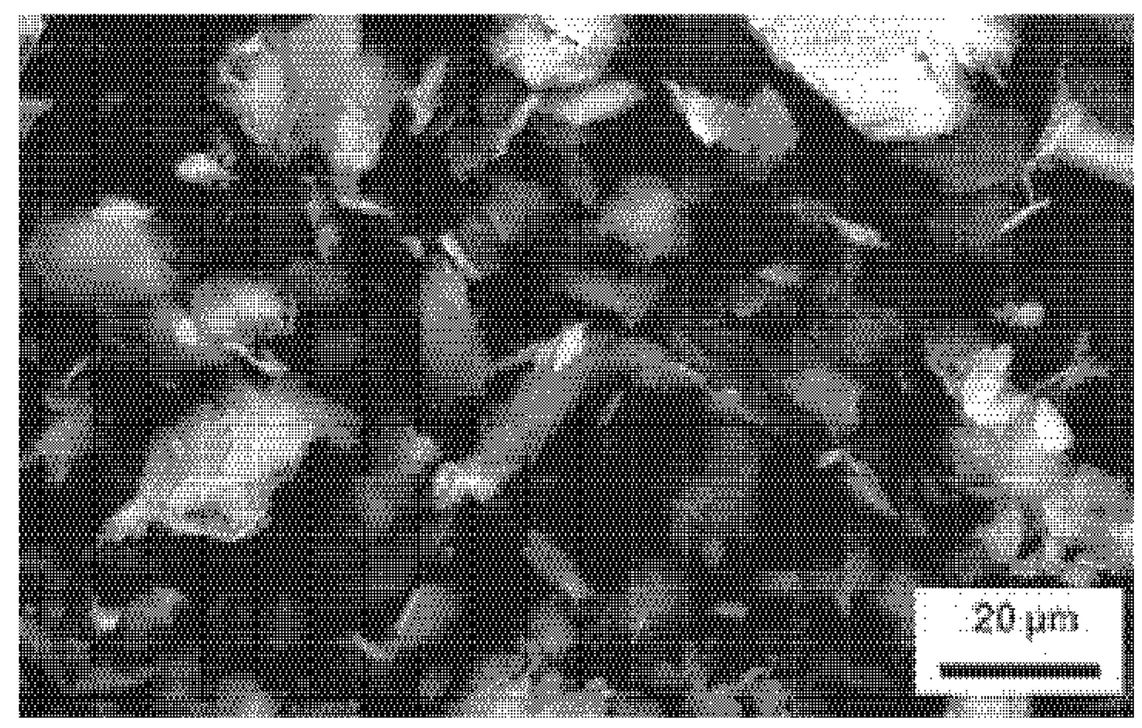

Figure 1. Scanning electron microscopy of the talc. Length of bar at right lower part in photo: 20 μm. Image was taken at 2000× magnification.

Material and Methods

Analysis of Talc Sample

Talc (nonasbestos form) was provided as ultra-fine white talcum powder from Rex Material (Korea) and was analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES). Structure and morphology of the particles were measured by field emission scanning electron microscopy (Quanta, Oregon) at an accelerating voltage of 20 kV. Talc samples were gold coated before measurement.

Animals and Treatments

Male and female 7-week-old Sprague-Dawley rats purchased from Orient Bio (Korea) were housed under standard laboratory conditions (temperature 22°C ± 3°C, humidity 50% ± 10%, and 12-hour day/night cycles). Animals were acclimatized to the facility for 1 week and observed for irregular behavior. Diets were provided ad libitum to the rats, except during exposure to talc. All animal experiments complied with the guidelines of the Institutional Animal Care and Use Committee. The rats were exposed to talc in a stainless steel inhalation chamber (Sibata, Japan) with a capacity of 1 m^3. Temperature and relative humidity were maintained at 22°C ± 3°C and 50% ± 20%, respectively. Talc aerosol was generated by a dust generator (Sibata) and was delivered into the chamber with air purified through a high-efficiency particulate air filter. The talc flow rate into the chamber was maintained by continuously controlling talc particle counts per minute. Talc concentrations in the chamber were measured by collecting samples with a SIP-32L sampler (Sibata). Groups of 6 male and 6 female rats were exposed to 0, 5, 50, and 100 mg/m^3 talc for 6 hours daily, 5 days/week for 4 weeks. Each rat was examined daily during exposure and postexposure to observe any symptoms related to talc exposure. After the terminal exposure, the rats were fasted for about 16 hours before necropsy.

Hematological and Biochemical Analyses

Blood samples were collected from the ventral aorta under isoflurane anesthesia. The blood samples were collected into complete blood count (CBC) bottles CBC bottles containing EDTA-2K and were analyzed using a Hemavet automatic hematology analyzer (Drew Scientific, Florida). The following parameters were determined: white blood cell count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophils count, red blood cell (RBC) count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin (MCH), MCH concentration, RBC distribution width, platelet (PLT) count, and mean plasma volume (MPV).

Blood samples for biochemical analyses were also collected from the ventral aorta in the plain tubes and were kept at room temperature so as to be clotted, and the sera were obtained by centrifugation of the blood samples at 1580 *g* for 10 minutes after clotting. Serum biochemistry parameters examined using a Drichem4000 automatic serum analyzer (Fuji Photo Film, Japan) were alkaline phosphatase, lactate dehydrogenase, GLU, total cholesterol, aspartate aminotransferase, triglyceride, alanine aminotransferase, urea nitrogen in blood, gamma-glutamyl transferase (GGT), albumin, total protein, creatinine, and total bilirubin.

Histopathological Examination

Lung tissues of 3 rats in each male and female group were fixed by inflation with 10% neutral buffered formalin. The tissues were embedded in paraffin, and sections 3 to 5 μm in thickness were placed on glass slides. After staining with hematoxylin and eosin (H&E), the tissue sections were examined using a light microscope (Olympus, Japan), and photomicrographs were taken with a built-in DP70 digital camera.

Western Blotting

Lungs of 3 rats in each male and female group were frozen with liquid nitrogen and ground to powder by mortar and pestle. The powder was sonicated in radioimmunoprecipitation assay tissue lysis buffer (Santa Cruz Biotechnology, Santa Cruz, California). Protein contents were determined by using the BCA protein assay kit (Thermo Scientific, Rockford, Illinois).

Table 1. Hematological Values of Male Rats Exposed to Talc for 4 Weeks by Inhalation.[a]

	Talc concentration (mg/m^3)			
Parameters	Control	5	50	100
WBC, $\times 10^9$/L	7.27 ± 1.57	6.32 ± 1.30	6.57 ± 2.79	7.43 ± 1.69
NE, $\times 10^9$/L	2.04 ± 0.46	1.78 ± 0.94	1.74 ± 0.13	2.51 ± 0.62
LY, $\times 10^9$/L	4.66 ± 1.08	4.02 ± 0.84	3.87 ± 1.55	4.48 ± 1.05
MO, $\times 10^9$/L	0.58 ± 0.19	0.70 ± 0.29	0.37 ± 0.27	0.40 ± 0.17
EO, $\times 10^9$/L	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.00	0.03 ± 0.02
BA, $\times 10^9$/L	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
RBC, $\times 10^{12}$/L	7.62 ± 0.49	7.56 ± 0.40	7.66 ± 0.41	7.39 ± 1.43
HB, g/dL	13.42 ± 0.99	13.62 ± 1.08	13.53 ± 0.61	13.80 ± 1.15
HCT, %	43.50 ± 3.71	42.50 ± 3.21	42.93 ± 1.22	44.13 ± 6.14
MCV, fL	57.10 ± 2.88	56.60 ± 1.89	56.10 ± 1.67	55.40 ± 1.98
MCH, pg	17.62 ± 0.85	17.53 ± 1.18	17.67 ± 0.67	16.83 ± 1.14
MCHC, g/dL	30.87 ± 1.16	31.05 ± 2.58	31.50 ± 0.70	30.43 ± 2.57
RDW, %	14.62 ± 0.84	14.28 ± 0.52	14.03 ± 0.60	14.68 ± 1.25
PLT, $\times 10^9$/L	919.80 ± 73.51	842.00 ± 50.71	821.00 ± 52.33	1108.00 ± 169.71
MPV, fL	6.27 ± 0.48	7.27 ± 0.54[b]	6.97 ± 0.06	6.53 ± 0.40

Abbreviations: WBC, white blood cell; NE, neutrophil; LY, lymphocyte; MO, monocyte; EO, eosinophil; BA, basophils; RBC, red blood cell; HB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; PLT, platelet; MPV, mean plasma volume.
[a]Size of each group (6).
[b]Significantly different with control ($P < 0.05$).

Proteins separated using 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis were transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, Massachusetts) for 2 hours at 400 mA. After transfer, the membrane was incubated in blocking solution (5% skim milk in phosphate buffered saline + 1% Tween, PBS-T), and primary antibody was applied for 2 hours. Primary antibodies used in this study were SOD2 (1:1000 dilution; Abcam, Cambridge, United Kingdom), GPx-1 (1:500 dilution; Abcam), and β-actin (1:1000 dilution; Cell Signaling Technology, Danvers, Massachusetts). The membrane was then incubated for 1 hour in a 1:2000 dilution of horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology). The membrane was washed by PBS-T 3 times, and target proteins were visualized using chemiluminescence (Pierce, Rockford, Illinois).

Statistical Analysis

All data are expressed as mean ± standard deviation (SD) unless otherwise specified. Statistical analyses were performed using SPSS version 12 (SPSS, Illinois). Statistical significance between the groups was analyzed by one-way analysis of variance followed by Tukey test. The level of statistical significance was $P < 0.05$.

Results

Chemical Composition, Structure, and Actual Concentration of the Talc

Talc contained 64.1% SiO_2, 32.6% MgO, 2.76% CaO, and 0.27% Na_2O, also including trace amounts of Fe_2O_3, Al_2O_3, and MnO. The crystal structure of talc particles was monoclinic and prismatic after analyzing by scanning electron microscopy (Figure 1). In addition, we confirmed that there was no asbestos in the talc sample we used in this study. Mass median aerodynamic diameter of talc aerosol generated in the chamber was 3.88 μm with a geometric SD of 1.86. Target concentration of talc in our study was 5, 50, and 100 mg/m^3 and we confirmed that the actual concentrations over the duration of the exposure period were 4.8 ± 0.7, 54.2 ± 7.5, and 101.5 ± 8.6 mg/m^3 at each of the exposure group.

Body Weight and Organ Weight Relative to Body Weight

There were no treatment-related adverse symptoms or deaths associated with inhaled talc during the experimental period. We measured body weight of experimental animal every 3 days. Body weight was slightly decreased on third day after exposure in 50 and 100 mg/m^3 exposure group of both male and female rats; however, there were no significance when compared with the control group (data not shown). There were no significant differences in the relative weight of the heart, thymus, kidney, liver, lung, and spleen compared to body weight (data not shown).

Hematological and Biochemical Analyses

Hematology evaluations for the male and female rats at the end of the study are shown in Tables 1 and 2, respectively. The MPV in males exposed to 5 mg/m^3 of talc and PLT count in females exposed to 50 mg/m^3 were increased significantly when compared with control rats. However, all hematological changes fell within the range of the historical control values.

Table 2. Hematological Values of Female Rats Exposed to Talc for 4 Weeks by Inhalation.[a]

	Talc concentration (mg/m^3)			
Parameters	Control	5	50	100
WBC, $\times 10^9$/L	4.90 ± 2.15	4.29 ± 1.12	5.27 ± 0.82	6.53 ± 0.49
NE, $\times 10^9$/L	1.34 ± 0.53	0.83 ± 0.23	1.03 ± 0.16	1.49 ± 0.22
LY, $\times 10^9$/L	3.64 ± 1.43	3.82 ± 0.24	4.32 ± 0.82	4.19 ± 1.37
MO, $\times 10^9$/L	0.40 ± 0.25	0.19 ± 0.06	0.27 ± 0.12	0.34 ± 0.20
EO, $\times 10^9$/L	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
BA, $\times 10^9$/L	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RBC, $\times 10^{12}$/L	6.98 ± 0.39	7.36 ± 0.18	7.22 ± 0.14	7.37 ± 0.50
HB, g/dL	12.52 ± 0.82	13.05 ± 0.58	12.67 ± 1.06	13.18 ± 1.32
HCT, %	40.18 ± 2.16	40.98 ± 0.48	41.37 ± 1.95	41.03 ± 2.15
MCV, fL	57.63 ± 2.19	55.70 ± 1.04	57.33 ± 1.83	56.02 ± 1.07
MCH, pg	17.95 ± 0.79	17.75 ± 0.62	17.53 ± 1.21	17.83 ± 0.74
MCHC, g/dL	31.17 ± 1.19	30.08 ± 4.01	30.60 ± 1.15	32.08 ± 1.56
RDW, %	12.90 ± 0.31	12.90 ± 0.64	12.93 ± 0.42	12.86 ± 0.53
PLT, $\times 10^9$/L	798.33 ± 50.93	910.00 ± 90.51	872.67 ± 23.69[b]	893.67 ± 13.87
MPV, fL	6.80 ± 0.60	6.76 ± 0.48	7.40 ± 0.85	7.40 ± 1.28

Abbreviations: WBC, white blood cell; NE, neutrophil; LY, lymphocyte; MO, monocyte; EO, eosinophil; BA, basophils; RBC, red blood cell; HB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; PLT, platelet; MPV, mean plasma volume.
[a]Size of each group (6).
[b]Significantly different with control (P < 0.05).

Table 3. Biochemical Serum Values in Male Rats Exposed to Talc for 4 Weeks by Inhalation.[a]

	Talc concentration (mg/m^3)			
Parameters	Control	5	50	100
ALP, mg/dL	439.50 ± 77.41	451.60 ± 68.07	527.20 ± 79.17	525.00 ± 75.45
LDH, U/L	808.20 ± 134.21	768.00 ± 188.10	890.00 ± 22.36	865.25 ± 59.31
GLU, mg/dL	199.00 ± 35.41	159.50 ± 31.63	136.80 ± 23.64[b]	146.17 ± 25.80[b]
TCHO, mg/dL	53.00 ± 16.79	53.50 ± 9.71	51.40 ± 8.08	54.00 ± 8.88
AST, U/L	90.00 ± 17.03	68.17 ± 11.65	122.00 ± 15.00	88.75 ± 25.55
TG, mg/dL	61.75 ± 15.61	58.80 ± 18.99	31.50 ± 5.00[b]	39.50 ± 9.33
ALT, U/L	25.67 ± 5.28	22.50 ± 3.51	25.00 ± 4.85	20.17 ± 0.98
BUN, mg/dL	14.20 ± 0.96	13.57 ± 1.23	14.52 ± 1.49	15.37 ± 1.63
GGT, U/L	8.67 ± 1.03	9.50 ± 0.84	7.40 ± 0.55	7.83 ± 0.98
ALB, g/dL	3.70 ± 0.13	3.60 ± 0.24	3.64 ± 0.18	3.65 ± 0.08
TP, g/dL	6.07 ± 0.30	5.82 ± 0.46	5.94 ± 0.25	6.10 ± 0.18
CRE, mg/dL	0.30 ± 0.06	0.25 ± 0.05	0.22 ± 0.04	0.27 ± 0.04
TBIL, mg/dL	0.43 ± 0.10	0.52 ± 0.18	0.48 ± 0.08	0.42 ± 0.04

Abbreviations: ALP, alkaline phosphatase; LDH, lactate dehydrogenase; GLU, glucose; TCHO, total cholesterol; AST, aspartate aminotransferase; TG, triglyceride; ALT, alanine aminotransferase; BUN, urea nitrogen in blood; GGT, gamma-glutamyl transferase; ALB, albumin; TP, total protein; CRE, creatinine; TBIL, total bilirubin.
[a]Size of each group (6).
[b]Significantly different with control (P < 0.05).

Glucose was decreased significantly in male rats exposed to 50 and 100 mg/m^3 (Table 3). Triglyceride was decreased in all male rats exposed to talc, with a significant difference only in the 50 mg/m^3 exposure group (Table 3). Aspartate aminotransferase and GGT were increased significantly in female rats exposed to 50 mg/m^3 compared with the control group (P < 0.05; Table 4). No other hematological and biochemical significant differences were evident in talc-exposed male and female rats.

Lung Histopathology

In male and female rats exposed to 50 and 100 mg/m^3 of talc, infiltration of foamy macrophages on the alveolar walls and spaces near the terminal and respiratory bronchioles occurred in a concentration-dependent manner (Figures 2 and 3). Interstitial pneumonitis, bronchial epithelial hyperplasia and hypertrophy, and arterial medial hypertrophy were observed in 1 male and female rat, respectively, with no relation to the exposure concentration (Table 5).

Table 4. Biochemical Serum Values in Female Rats Exposed to Talc for 4 Weeks by Inhalation.[a]

	Talc concentration (mg/m^3)			
Parameters	Control	5	50	100
ALP, mg/dL	292.40 ± 39.97	397.00 ± 103.52	375.20 ± 64.55	347.75 ± 93.25
LDH, U/L	853.00 ± 94.00	883.33 ± 28.87	891.67 ± 20.41	890.00 ± 22.36
GLU, mg/dL	106.20 ± 21.23	140.50 ± 19.12	112.33 ± 27.29	118.20 ± 26.53
TCHO, mg/dL	72.40 ± 19.15	77.50 ± 17.08	78.00 ± 14.52	73.00 ± 16.61
AST, U/L	71.17 ± 12.09	69.00 ± 20.51	117.80 ± 14.32[b]	95.75 ± 8.66
TG, mg/dL	36.60 ± 7.99	25.75 ± 7.89	30.60 ± 10.21	26.00 ± 2.83
ALT, U/L	18.50 ± 3.89	17.75 ± 3.40	17.50 ± 3.89	17.60 ± 3.78
BUN, mg/dL	14.30 ± 1.14	15.70 ± 2.20	14.75 ± 1.79	14.10 ± 1.71
GGT, U/L	8.50 ± 0.55	8.50 ± 0.58	10.83 ± 1.83[b]	8.60 ± 1.52
ALB, g/dL	4.17 ± 0.23	4.20 ± 0.22	4.23 ± 0.25	4.20 ± 0.24
TP, g/dL	6.80 ± 0.34	6.58 ± 0.59	6.78 ± 0.42	6.84 ± 0.59
CRE, mg/dL	0.33 ± 0.05	0.28 ± 0.05	0.30 ± 0.06	0.28 ± 0.04
TBIL, mg/dL	0.48 ± 0.04	0.60 ± 0.24	0.68 ± 0.21	0.60 ± 0.07

Abbreviations: ALP, alkaline phosphatase; LDH, lactate dehydrogenase; GLU, glucose; TCHO, total cholesterol; AST, aspartate aminotransferase; TG, triglyceride; ALT, alanine aminotransferase; BUN, urea nitrogen in blood; GGT, gamma-glutamyl transferase; ALB, albumin; TP, total protein; CRE, creatinine; TBIL, total bilirubin.
[a]Size of each group (6).
[b]Significantly different with control ($P < 0.05$).

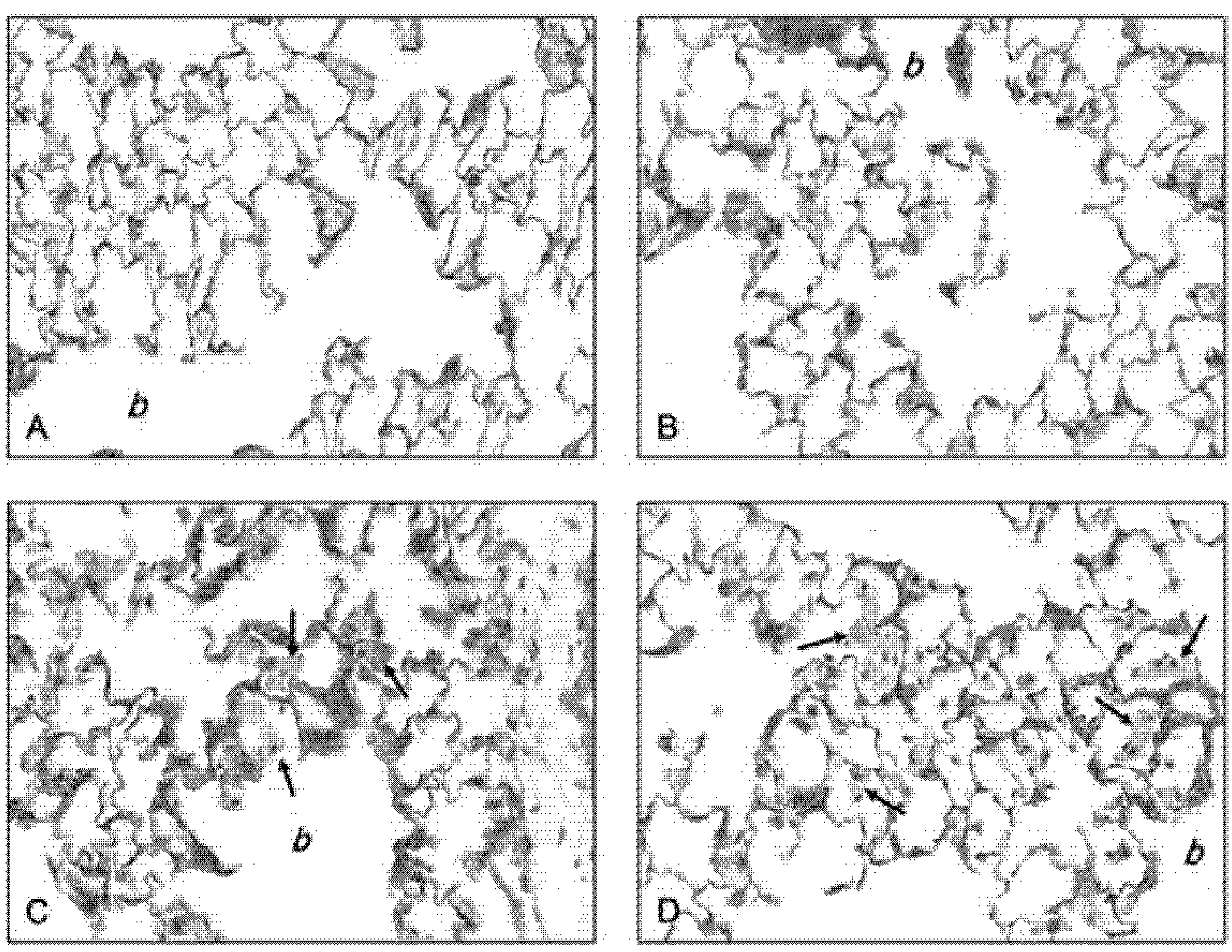

Figure 2. Histopathological findings in the lungs of male rats after exposure to talc for 4 weeks. A, Control and (B) low (50 mg/m^3) exposure group. In the middle (C, 50 mg/m^3) and high (D, 100 mg/m^3) exposure group, the foamy macrophages (arrows) were infiltrated on the alveolar walls and spaces near the terminal bronchioles (*b*). After hematoxylin and eosin (H&E) staining, the image was taken at 400× magnification.

Expression of SOD2 and GPx1 in Lung

A significant fold-induction of SOD2 was evident between the control group and both the low and the high talc inhalation groups of male rats ($P < 0.05$). Expression of SOD2 in lungs of male rats exposed to the highest concentration of talc was more than twice the level in control rats (Figure 4). Expression of SOD2 in the female rats who inhaled the highest concentration of talc was 4 times that of the control group. Changes in the

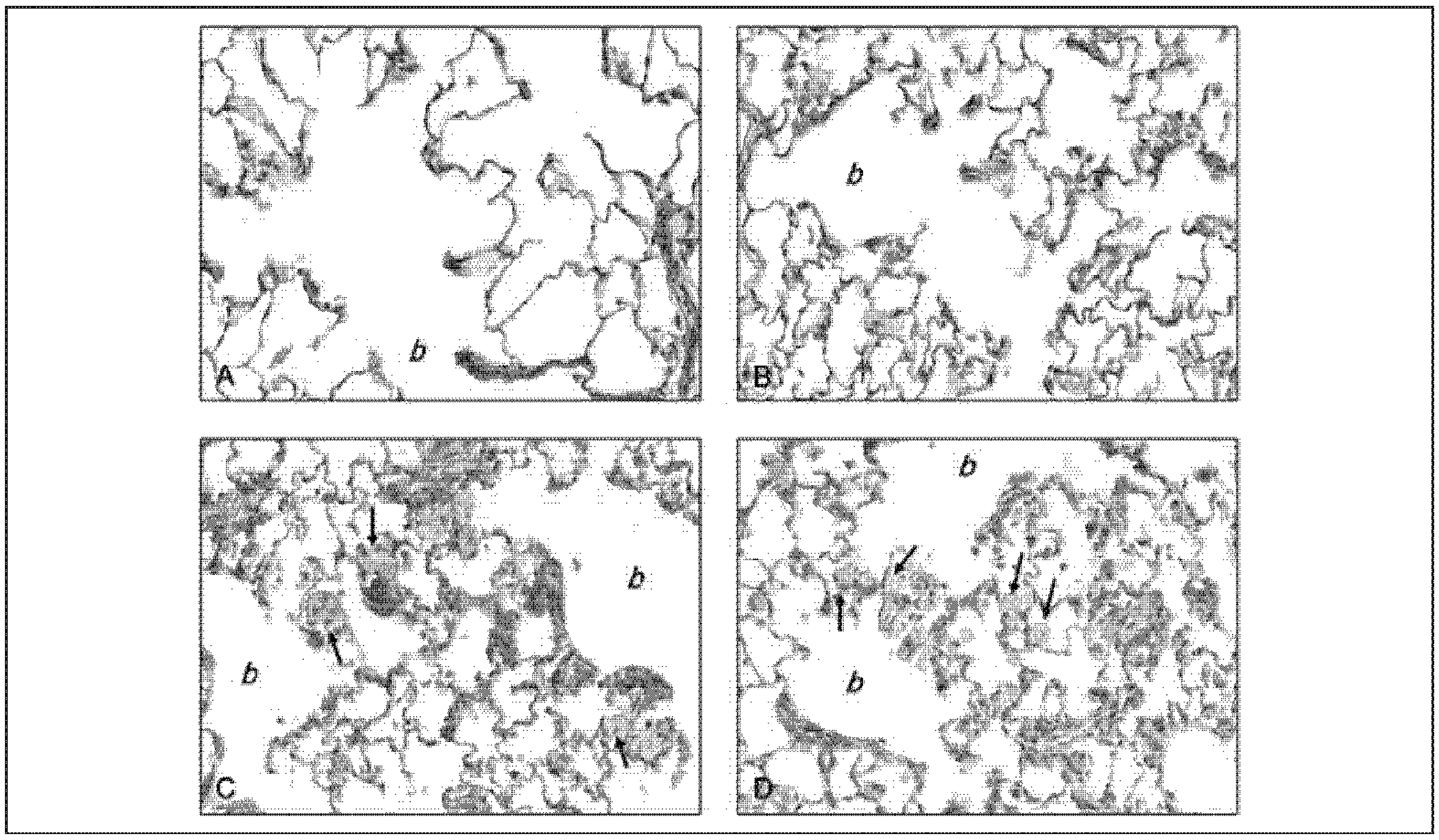

Figure 3. Histopathological findings in the lungs of female rats after exposure to talc for 4 weeks. A, Control and (B) low (50 mg/m^3) exposure group. Note the foamy macrophages (arrows) infiltrated on the alveolar walls and spaces near the terminal bronchioles (*b*) in the middle (C, 50 mg/m^3) and high (D, 100 mg/m^3) exposure group. After hematoxylin and eosin (H&E) staining, the image was taken at 400× magnification.

Table 5. Incidence of Histopathological Findings in the Lungs of the Rats Exposed to Talc.

Sex	Male				Female			
Talc concentration (mg/m^3)	**0**	**5**	**50**	**100**	**0**	**5**	**50**	**100**
Number of rats examined	**3**	**3**	**3**	**3**	**3**	**3**	3	**3**
No specific lesion	**3**	**3**	**0**	**0**	**3**	**2**	**0**	**0**
Foamy histiocytes	**0**	**0**	**3**	**3**	**0**	**0**	3	**3**
Grades: Minimal	0	0	2	0	0	0	3	0
Mild	0	0	1	2	0	0	0	3
Moderate	0	0	0	1	0	0	0	0
Interstitial pneumonitis, diffuse	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**0**
Grades: Minimal	0	0	0	0	0	1	0	0
Mild	0	0	0	1	0	0	0	0
Bronchial epithelial hyperplasia and hypertrophy	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**0**
Grades: Minimal	0	0	0	0	0	1	0	0
Mild	0	0	0	1	0	0	0	0
Arterial medial hypertrophy	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
Grades: Minimal	0	0	0	0	0	0	0	0
Mild	0	0	0	1	0	0	0	0

expression of GPx1 in male rats who inhaled the lowest and highest concentrations of talc were more than double those of control rats. The difference between the control group and low exposed group was significant. Glutathione peroxidase 1 tended to be increased in all females exposed to talc, albeit without statistical significance. Overall, SOD2 was a more significant oxidative stress marker than GPx1 in the lungs of rats exposed to talc.

Discussion

Talc is a white or gray fine powder that is acid- and temperature-resistant and water insoluble. A great amount of talc has been used occupationally, for example, in the rubber industry and could be exposed to human in our daily life when people use cosmetic products such as baby powder.[35] In addition, exposure of hamsters to an aerosol of cosmetic talc led to deposition of particles in the lung.[36]

The present study was performed to evaluate 4-week pulmonary toxicity of inhaled talc including the expression of antioxidant enzymes in lung. Male and female rats were exposed to different concentrations of talc for 4 weeks. The composition of talc used in this study was determined, and hematological, biochemical, and histopathological comparisons with control rats were performed. In addition, the effects of inhaled talc on antioxidant enzymes in lung were evaluated.

Analysis of chemical composition of talc by ICP-AES showed that the talc sample used in this study contained mainly SiO_2 and MgO. This finding is consistent with the prior report revealing that talc comprised 63.4% SiO_2, 31.9% MgO, and 4.8% water.[37] SiO_2 was highly toxic to alveolar macrophages in humans and rats, generating free radical-mediated oxidative

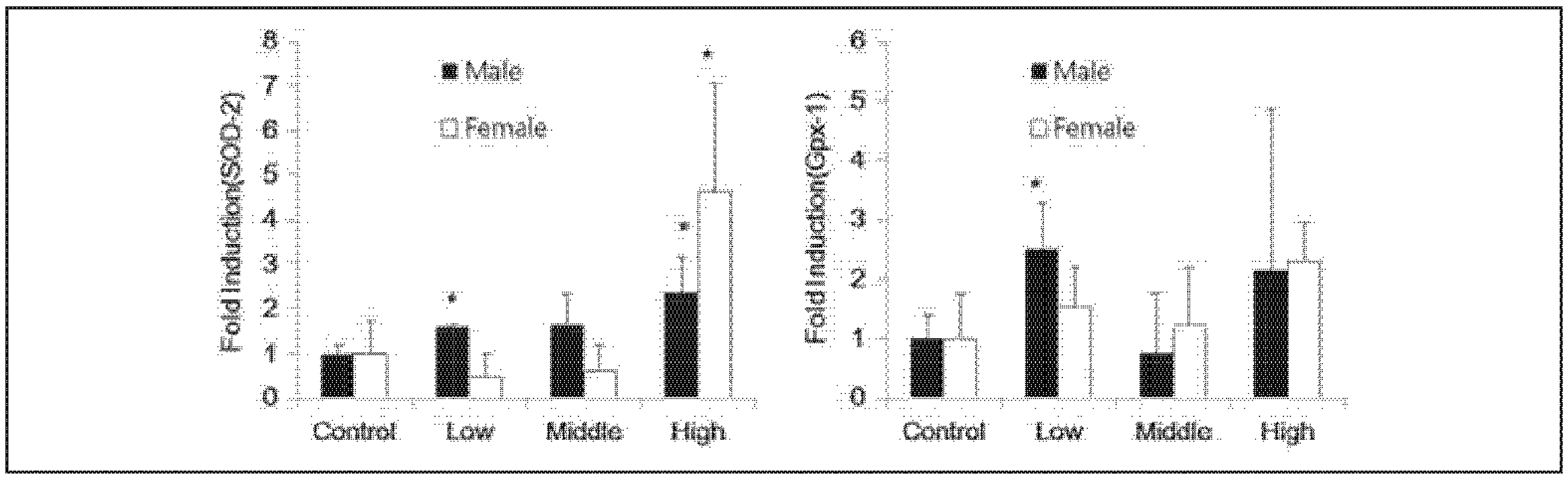

Figure 4. Expression of superoxide dismutase 2 (SOD2) and glutathione peroxidase (GPx1) in the lungs of male and female rats after exposure to talc for 4 weeks. *$P < 0.05$ versus control group.

stress and inducing tumor necrosis factor, one of cytokine.[38] Numerous studies on SiO_2, especially nano-sized particle, revealed that it caused various pulmonary diseases, oxidative stress, and lipid peroxidation.[39]

Particles deposited in the alveolar region are phagocytized by alveolar macrophages. The present histopathological examination revealed foamy macrophage infiltrates in the lungs of male and female rats exposed to 50 and 100 mg/m^3 talc, indicating that macrophage infiltration was a direct consequence of the inhalation of talc. Macrophage infiltration was found on the alveolar walls and spaces near the terminal bronchioles, which is the route of inhaled particles. Pickrell et al reported that repeated inhalation exposure of rats to talc increased the number of intra-alveolar macrophages.[24] Another talc inhalation study using 4-week-old hamsters revealed lung interstitial pneumonia, alveolar emphysema, alveolar and bronchiolar calcification, alveolar hyperplasia, and alveolar histiocytosis.[36]

Exposure to inhaled particles in experimental animals could lead to lung burdens and alveolar inflammation. The inflammatory response might give rise to synthesis of reactive oxygen species and cell injuries.[40] In biological systems, antioxidant enzymes defend cells against various oxidative stresses. We examined the protein expression changes of SOD2 (also called manganese SOD) and GPx1 (also called cytosolic GPx), which are oxidative stress responsive enzymes. Superoxide dismutases catalyze superoxide anions into hydrogen peroxide efficiently.[41] In the present study, SOD2 was induced significantly in male rats exposed to low and high concentrations of inhaled talc and in female rats exposed to the high inhaled concentration. These results were consistent with the previous research, revealing that inhaled cristobalite and TiO_2 particles increased SOD2 immunoreactive protein.[42] Another previous study showed that SOD2 and GPx1, representative enzymes associated with oxidative stress response, were significantly upregulated when rats exposed to manufactured ultrafine particles.[43] Glutathione peroxidase 1 detoxifies peroxides with reduced form of GSH, thiol-containing tripeptide, acting as an electron donor, with the production of glutathione disulfide.[44] In the present study, GPx1 tended to increase in rat lungs exposed to talc, albeit not with appreciable statistical significance. Superoxide dismutase 2 was induced significantly more than GPx1 did in rat lungs exposed to talc; therefore, we concluded that SOD2 was more sensitive oxidative stress indicator than GPx1 in this study. Lung tissues of only 3 rats in each male and female group were used in this study on the induction of enzyme, therefore, we might have the experimental limitation for getting significant results of concentration-dependent induction of antioxidant enzymes. It is well known that cellular defense against free radicals is related to various antioxidant enzymes such as catalase, glutathione reductase, and glutamate-cysteine ligase, in addition to GPx and SOD.[45] Further studies on induction of all these enzymes are necessary to understand the antioxidant activities in rats exposed to talc comprehensively.

The decrease of GLU in the male rats might be related to the decreased body weight. There were no treatment-related symptoms or mortality in any of the rats treated with talc during the study period. Histopathological examination revealed that macrophage infiltration was observed on the alveolar walls and spaces near the terminal and respiratory in the 50 mg/m^3 and 100 mg/m^3 exposure groups. In addition, in male and female rats exposed to 100 mg/m^3 talc, SOD2 expression was significantly increased ($P < 0.05$). Taken together, these results demonstrate that 4 weeks repeated inhalation of talc in rats could induce macrophage aggregations around the terminal airways and oxidative damage in the lung.

Acknowledgments

This study was supported by the National Institute of Environmental Research, Republic of Korea.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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Doc ID: J0163977 Version:0.4 Status:Draft

Office of Consumer Medical Safety

Analysis of Post-Marketing Safety Reports in RSS Global Safety Database

Johnson's® Baby Powder, Shower to Shower® Powder

Status: Final
Date: 15 March 2016
Prepared by: Office of Consumer Medical Safety
Document No.: J No. J0163936

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APPROVALS

I have reviewed this report and confirm that, to the best of my knowledge, it accurately describes the data available to date:

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Doc ID: J0163977 Version:0.4 Status:Draft

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1. INTRODUCTION

On 25 February 2016, the United States (US) Food and Drug Administration (FDA) requested to provide all safety literature and data regarding talc.

For the purpose of this report the term "case" represents an individual safety report. Cases received from spontaneous notification, including reports from healthcare professionals, consumers, scientific literature, regulatory authorities and other sources are included in the global safety database Remetrex Safety System (RSS).

This report presents the results of the review of post-marketing cases concerning talc containing powder products Johnson's® Baby Powder and Shower to Shower® Powder.

2. METHODS

2.1. Remetrex Safety System Global Safety Database Search

A search of Remetrex Safety System (RSS) Global Safety Database was performed for all cases that met the following criteria:

- Closed cases only
- Cases reported cumulatively through 23 February 2016
- Medically assessed cases with AE Medical Dictionary for Regulatory Activities (MedDRA) coding
- List of Company products Johnson's® Baby Powder or Shower to Shower® Powder (see Attachment 1) searched regardless of drug role (suspect or suspect-interacting or concomitant)
- Medically confirmed and medically unconfirmed cases
- All type of cases (eg spontaneous/clinical study/registry etc.)
- Version of case: highest version

Please note that in RSS database processing of cases received from North America began in September 2011 and the processing of cases received from rest of the world started in July 2014. Due to pragmatic reasons (current availability) only data available in RSS Global Safety Database is reviewed in this report.

All cases retrieved by the search were reviewed and analyzed according to the methods described in Section 2.2, Data Review and Analysis.

2.2. Data Review and Analysis

The cases were reviewed in aggregate and results are presented based on MedDRA System Organ Class Groups. Cases concerning System Organ Class (SOC) Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps) are presented in more detail due to recent special interest in this topic.

2.3. Cases from the RSS Global Safety Database

The review and analysis of cases from the RSS Global Safety Database were conducted using a step-wise approach.

- Initial aggregate data analysis: The retrieved cases were categorized as per SOC. Cases pertaining to all the SOCs were analyzed and stratified by patient demographics (sex, age, and country of origin) and case characteristics (seriousness, medical confirmation, and outcome). A case may report an event more than once with respect to its association to more than 1 suspect product. In such instances, the event was counted only once to determine its frequency. Percentages were calculated using unique case counts.
- Individual case-level review: Cases pertaining to the SOCs Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps) and Respiratory, Thoracic and Mediastinal Disorders were reviewed in their entirety for a potential drug-effect relationship in accordance with the CIOMS Threshold Criteria 1a: "Evidence from Individual Cases." With respect to the remaining SOCs, individual case-level review was performed for cases assessed as serious.
- Trend analysis/Cumulative weight of evidence: After reviewing all cases, an assessment of the cumulative weight of evidence was performed taking into account all available evidence from the post-marketing cases, and applying CIOMS Threshold Criteria I, II, III, and IV.

3. RESULTS

3.1. Review of Cases from RSS Global Safety Database

The cases were reviewed and results are presented below based on MedDRA SOC Groups. Cases concerning SOC Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps) are presented in more detail due to recent special interest in this topic.

3.1.1. SOC General Disorders and Administration Site Conditions

3.1.1.1. Johnson's® Baby Powder

3.1.1.1.1. Case Screening Results

The search of the RSS Global Safety Database retrieved 1296 total cases reporting the use of Johnson's® Baby Powder for PTs applicable to the General Disorders and Administration Site Conditions SOC. The distribution of the MedDRA PTs is presented in Table 1.

Table 1: Preferred Terms in Cases Reporting Use of Johnson's® Baby Powder Representing SOC General Disorders and Administration Site Conditions (n=1296)

MedDRA PTs	Number of PTs[a] (%)
Application site rash	505 (39)
Application site erythema	173 (13.3)
Therapeutic response unexpected	165 (12.7)
Application site pruritus	154 (11.9)
No adverse event	105 (8.1)
Application site irritation	85 (6.6)
Product odour abnormal	76 (5.9)

Table 1: Preferred Terms in Cases Reporting Use of Johnson's® Baby Powder Representing SOC General Disorders and Administration Site Conditions (n=1296)

MedDRA PTs	Number of PTs[a] (%)
Application site pain	67 (5.2)
Application site papules	66 (5.1)
Application site exfoliation	48 (3.7)
Application site dryness	45 (3.5)
Application site acne	42 (3.2)
Application site reaction	38 (2.9)
Product lot number issue	38 (2.9)
Suspected counterfeit product	35 (2.7)
Product quality issue	34 (2.6)
Application site urticaria	32 (2.5)
Application site vesicles	26 (2)
Application site discolouration	25 (1.9)
Product container issue	21 (1.6)
Application site swelling	17 (1.3)
Discomfort	14 (1.1)
Application site warmth	13 (1)
Drug ineffective	13 (1)
Application site erosion	11 (0.8)
Drug ineffective for unapproved indication	11 (0.8)
Application site haemorrhage	10 (0.8)
Product packaging issue	10 (0.8)
Malaise	9 (0.7)
Product label issue	9 (0.7)
Product physical issue	9 (0.7)
Application site hypersensitivity	8 (0.6)
Application site scab	8 (0.6)
Application site burn	7 (0.5)
Product container seal issue	6 (0.5)
Product expiration date issue	6 (0.5)
Application site eczema	5 (0.4)
Application site inflammation	5 (0.4)
Application site paraesthesia	5 (0.4)
Product counterfeit	5 (0.4)
Application site discharge	4 (0.3)
Application site scar	4 (0.3)
Product contamination physical	4 (0.3)
Adverse drug reaction	3 (0.2)
Application site discomfort	3 (0.2)
Application site alopecia	2 (0.2)
Application site odour	2 (0.2)
Death	2 (0.2)
Drug effect incomplete	2 (0.2)
Product physical consistency issue	2 (0.2)
Administration site rash	1 (0.1)
Adverse event	1 (0.1)
Application site coldness	1 (0.1)
Application site dermatitis	1 (0.1)
Application site ulcer	1 (0.1)
Crying	1 (0.1)
Drug effect decreased	1 (0.1)
Pain	1 (0.1)
Product contamination microbial	1 (0.1)
Product label counterfeit	1 (0.1)

Table 1: Preferred Terms in Cases Reporting Use of Johnson's® Baby Powder Representing SOC General Disorders and Administration Site Conditions (n=1296)

MedDRA PTs	Number of PTs[a] (%)
Product outer packaging issue	1 (0.1)
Product packaging quantity issue	1 (0.1)
Product taste abnormal	1 (0.1)
Pyrexia	1 (0.1)
Swelling	1 (0.1)

Key: MedDRA=Medical Dictionary for Regulatory Activities; n=Number of Cases; PT=Preferred Term; SOC=System Organ Class
a: Cases may contain more than 1 PT.

3.1.1.1.2. Case Demographics

Of the 1296 cases, the ages of the patients were reported in 186 cases. The mean age was 27.6 years, median was 3.5 years, and the range was 1 week to 85 years.

The patient demographics by gender and age, and the country of origin of the cases retrieved are provided in Table 2. Males accounted for 33.1% (428/1296) of the 1296 cases, while females accounted for 52.8% (684/1296). The gender was not reported in 14.1% (184/1296) of the cases. Cases were received from 15 countries. The leading reporting country for cases pertaining to this topic was the US accounting for 66.4% (861/1296) of the 1296 cases. The second leading reporting country was China accounting for 13% (168/1296) of the cases.

Table 2: Patient Demographics of Cases Concerning Johnson's® Baby Powder Representing SOC General Disorders and Administration Site Conditions (n=1296)

Characteristic		Number of Cases (%)
Gender	Female	684 (52.8)
	Male	429 (33.1)
	Not reported	183 (14.1)
Patient Age (years)	0 to <2	76 (5.9)
	2 to <12	19 (1.5)
Mean: 27.6	12 to <18	2 (0.2)
Median: 3.5	18 to <35	8 (0.6)
Range: 1 week to	35 to <50	19 (1.5)
85 years	50 to <65	36 (2.8)
	≥65	26 (2)
	Not reported	1110 (85.6)

Table 2: Patient Demographics of Cases Concerning Johnson's® Baby Powder Representing SOC General Disorders and Administration Site Conditions (n=1296)

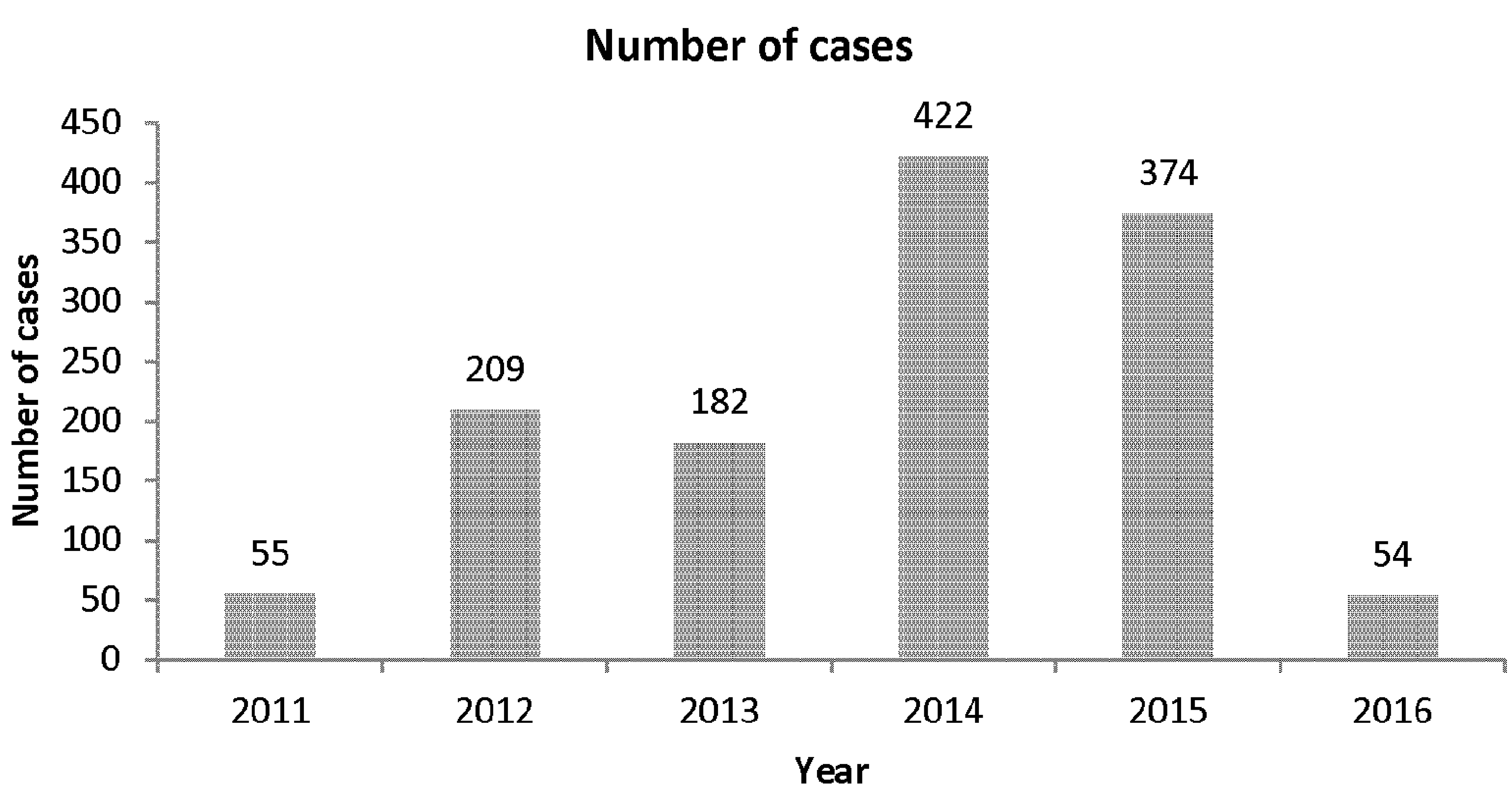
Characteristic		Number of Cases (%)
Country of Origin	United States	861 (66.4)
	China	168 (13)
	India	114 (8.8)
	Canada	72 (5.6)
	Australia	25 (1.9)
	Viet Nam	14 (1.1)
	Japan	13 (1.0)
	Indonesia	6 (0.5)
	Thailand	6 (0.5)
	Korea, Republic Of	5 (0.4)
	New Zealand	4 (0.3)
	Trinidad And Tobago	3 (0.2)
	Poland	2 (0.2)
	Taiwan	2 (0.2)
	Philippines	1 (0.1)

Key: n=Number of Cases; SOC=System Organ Class

3.1.1.1.3. Trending of Cases Representing SOC General Disorders and Administration Site Conditions

Figure 1 represents the number of cases reporting the use of Johnson's® Baby Powder for PTs applicable to the General Disorders and Administration Site Conditions SOC. The highest number of cases was received in the year 2014.

Figure 1: Trend Analysis of Cases Concerning Johnson's® Baby Powder Representing SOC General Disorders and Administration Site Conditions*



*Data cut-off date is 23 February 2016

3.1.1.1.4. Case Characteristics

The following characteristics of the 1296 cases were analysed and tabulated: seriousness, medical confirmation, and outcome.

Table 3 below describes the case criteria break down of the 1296 cases into case level seriousness, medical confirmation, and case outcome. The majority of cases (98.8% [1281/1296]) were categorised as medically unconfirmed.

Table 3: Seriousness, Medical Confirmation, and Outcome of Cases Concerning Johnson's® Baby Powder Representing SOC General Disorders and Administration Site Conditions (n=1296)

Characteristic	Number of Cases (%)
Seriousness	
Serious	10 (0.8)
Nonserious	1286 (99.2)
Medically Confirmed	
Yes	14 (1.1)
No	1281 (98.8)
Not reported	1 (0.1)
Outcome	
Recovered	263 (20.3)
Not recovered	244 (18.8)
Recovering	120 (9.3)
Event ongoing	12 (0.9)
Not reported	657 (50.7)
Total Number of Cases	**1296**

Key: n=Number of Cases; SOC=System Organ Class

Table 4 provides an overview of the serious AEs (SAEs) reported in 10 cases.

Table 4: Serious Adverse Events in Cases Johnson's® Baby Powder Representing SOC General Disorders and Administration Site Conditions (n=10)

MedDRA PTs	Number of PTs[a] (%)
Application site erythema	2 (20)
Application site rash	2 (20)
Death	2 (20)
Application site erosion	1 (10)
Application site haemorrhage	1 (10)
Application site irritation	1 (10)
Application site pain	1 (10)
Application site papules	1 (10)
Application site pruritus	1 (10)
Application site scar	1 (10)
Application site swelling	1 (10)
Product lot number issue	1 (10)
Suspected counterfeit product	1 (10)

Key: n=Number of Cases; PT=Preferred Term; SOC=System Organ Class
a: Cases may contain more than 1 PT.

Of these 10 serious cases, 2 cases reported a fatal outcome. The cases reported insufficient information regarding the course of the events for an adequate medical assessment.

Of the remaining 8 serious cases, 3 cases reported events which may have resulted due to a hypersensitivity reaction to the product. The remaining 5 cases reported insufficient information regarding dose, time to onset, medical history and concomitant medications for a meaningful medical assessment.

Overall, based on the review of the cases no new safety concerns were identified.

3.1.1.2. Shower to Shower® Powder

3.1.1.2.1. Case Screening Results

The search of the RSS Global Safety Database retrieved 31 total cases reporting the use of Shower to Shower® Powder for PTs applicable to the General Disorders and Administration Site Conditions SOC. The distribution of the PTs is presented in Table 5.

Table 5: Preferred Terms in Cases Concerning Shower to Shower® Powder Representing SOC General Disorders and Administration Site Conditions (n=31)

MedDRA PTs	Number of PTs[a] (%)
Application site rash	13 (41.9)
Application site pruritus	12 (38.7)
Application site erythema	4 (12.9)
Application site pain	4 (12.9)
Application site papules	3 (9.7)
Product odour abnormal	3 (9.7)
Product quality issue	3 (9.7)
Therapeutic response unexpected	3 (9.7)
Adverse drug reaction	1 (3.2)
Application site irritation	1 (3.2)
Application site reaction	1 (3.2)
Application site swelling	1 (3.2)
Application site urticaria	1 (3.2)
Application site warmth	1 (3.2)
Malaise	1 (3.2)
Product container issue	1 (3.2)

Key: MedDRA=Medical Dictionary for Regulatory Activities; n=Number of Cases; PT=Preferred Term; SOC=System Organ Class
a: Cases may contain more than 1 PT.

3.1.1.2.2. Case Demographics

Of the 31 cases, the ages of the patients were reported in 16 cases. The mean age was 45.2 years, median was 40 years, and the range was 19 to 82 years.

The patient demographics by gender and age, and the country of origin of the cases retrieved are provided in Table 6. Females accounted for 74.2% (23/31) of the 31 cases, while males accounted for 25.8% (8/31). The gender was reported in all the cases. All the cases were received from 1 country, the US.

Table 6: Patient Demographics of Cases Concerning Shower to Shower® Powder Representing SOC General Disorders and Administration Site Conditions (n=31)

Characteristic		Number of Cases (%)
Gender	Female	23 (74.2)
	Male	8 (25.8)

Table 6: Patient Demographics of Cases Concerning Shower to Shower® Powder Representing SOC General Disorders and Administration Site Conditions (n=31)

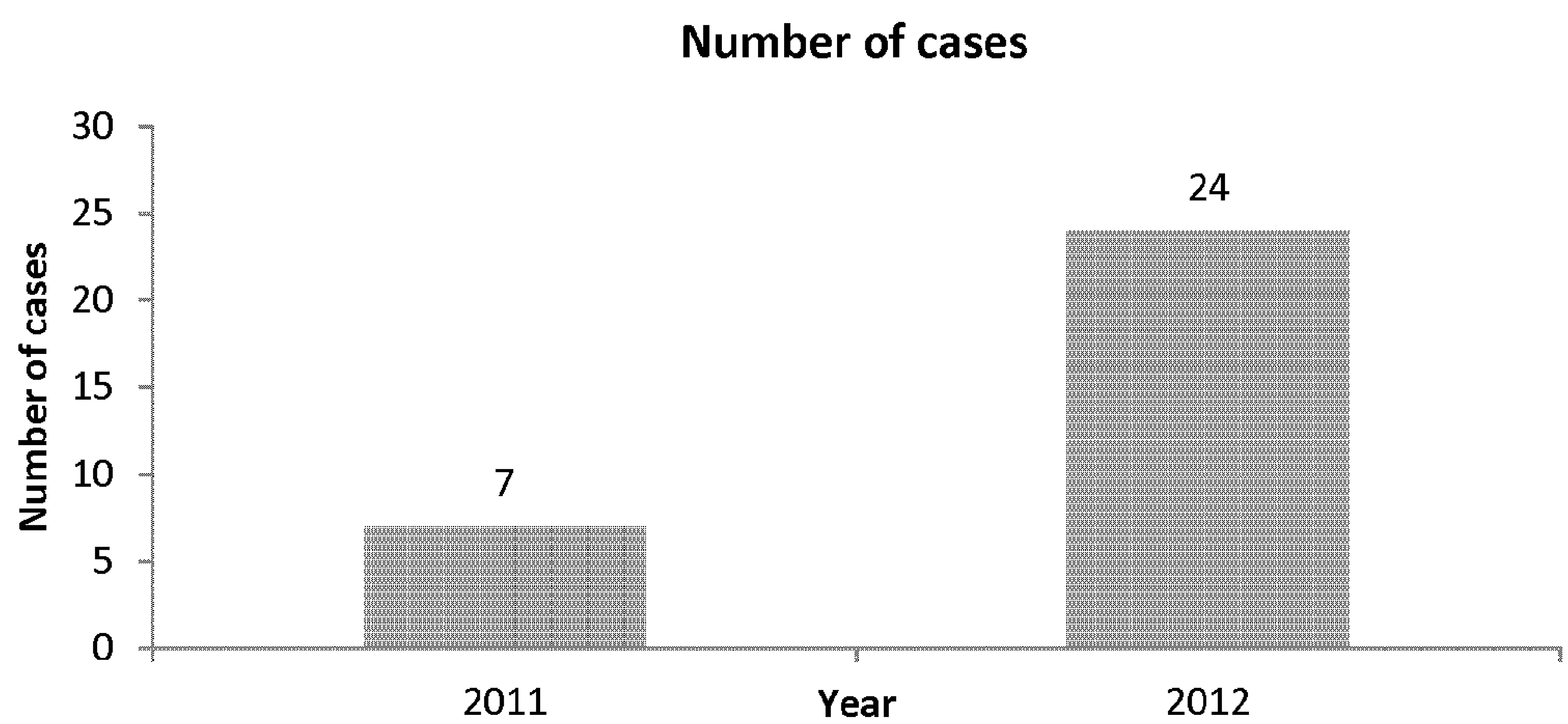
Characteristic		Number of Cases (%)
Patient Age (years)	18 to <35	6 (19.4)
	35 to <50	4 (12.9)
Mean: 45.2	50 to <65	3 (9.7)
Median: 40	≥65	3 (9.7)
Range: 19 to 82	Not reported	15 (48.4)
Country of Origin	United States	31 (100)

Key: n=Number of Cases; SOC=System Organ Class

3.1.1.2.3. Trending of Cases Representing SOC General Disorders and Administration Site Conditions

Figure 2 represents the number of cases reporting the use of Shower to Shower® Powder for PTs applicable to the General Disorders and Administration Site Conditions SOC. The comparatively higher number of cases was received in the year 2012, post which no case was received concerning SOC General Disorders and Administration Site Conditions.

Figure 2: Trend Analysis of Cases Concerning Shower to Shower® Powder Representing SOC General Disorders and Administration Site Conditions*



*Data cut-off date is 23 February 2016

3.1.1.2.4. Case Characteristics

The following characteristics of the 31 cases were analysed and tabulated: seriousness, medical confirmation, and outcome.

Table 7 below describes the case criteria break down of the 31 cases into case level seriousness, medical confirmation, and case outcome.

Table 7: Seriousness, Medical Confirmation, and Outcome of Cases Concerning Shower to Shower® Powder Representing SOC General Disorders and Administration Site Conditions (n=31)

Characteristic	Number of Cases (%)
Seriousness	
Nonserious	31 (100)
Medically Confirmed	
Yes	1 (3.2)
No	30 (96.8)
Outcome	
Recovered	15 (48.4)
Not Recovered	10 (32.3)
Recovering	2 (6.5)
Not reported	4 (12.9)
Total Number of Cases	**31**

Key: n=Number of Cases; SOC=System Organ Class

All cases reported nonserious events. The majority of cases (96.8% [30/31]) were categorised as medically unconfirmed. Overall, no new significant safety concern was identified.

3.1.2. SOC Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps)

3.1.2.1. Johnson's® Baby Powder

3.1.2.1.1. Case Screening Results

The search of the RSS Global Safety Database retrieved 1269 total cases for PTs applicable to the Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps) SOC. The distribution of the PTs is presented in Table 8.

Table 8: Preferred Terms in Cases Representing SOC Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps) (n=1269)

MedDRA PTs	Number of PTs[a] (%)
Ovarian cancer	929 (73.2)
Ovarian cancer stage III	140 (11)
Ovarian cancer stage I	68 (5.3)
Ovarian cancer stage IV	43 (3.4)
Ovarian cancer stage II	40 (3.2)
Ovarian cancer metastatic	20 (1.6)
Endometrial adenocarcinoma	9 (0.7)
Uterine cancer	7 (0.6)
Fallopian tube cancer stage III	6 (0.5)
Cervix carcinoma	4 (0.3)
Endometrial cancer stage III	3 (0.2)
Fallopian tube cancer	3 (0.2)
Malignant peritoneal neoplasm	3 (0.2)
Neoplasm malignant	3 (0.2)
Ovarian cancer recurrent	3 (0.2)
Basal cell carcinoma	2 (0.2)
Endometrial cancer stage II	2 (0.2)
Fallopian tube cancer stage I	2 (0.2)
Mesothelioma malignant	2 (0.2)

Table 8: Preferred Terms in Cases Representing SOC Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps) (n=1269)

MedDRA PTs	Number of PTs[a] (%)
Adenocarcinoma	1 (0.1)
Borderline serous tumour of ovary	1 (0.1)
Brain neoplasm malignant	1 (0.1)
Breast cancer	1 (0.1)
Breast cancer female	1 (0.1)
Breast cancer metastatic	1 (0.1)
Carcinoid tumour	1 (0.1)
Cervix carcinoma stage 0	1 (0.1)
Clear cell endometrial carcinoma	1 (0.1)
Colon cancer stage IV	1 (0.1)
Fallopian tube cancer stage II	1 (0.1)
Laryngeal cancer	1 (0.1)
Leiomyosarcoma	1 (0.1)
Lung neoplasm malignant	1 (0.1)
Melanoma recurrent	1 (0.1)
Metastases to central nervous system	1 (0.1)
Metastases to lymph nodes	1 (0.1)
Neoplasm of appendix	1 (0.1)
Non-Hodgkin's lymphoma	1 (0.1)
Ovarian granulosa cell tumour	1 (0.1)
Pancreatic carcinoma	1 (0.1)
Papillary serous endometrial carcinoma	1 (0.1)
Pelvic neoplasm	1 (0.1)
Peritoneal carcinoma metastatic	1 (0.1)
Pleural mesothelioma malignant	1 (0.1)
Prostate cancer	1 (0.1)
Recurrent cancer	1 (0.1)
Skin cancer	1 (0.1)
Squamous cell carcinoma of the cervix	1 (0.1)
Uterine leiomyoma	1 (0.1)
Vaginal cancer stage I	1 (0.1)

Key: MedDRA=Medical Dictionary for Regulatory Activities; n=Number of Cases; PT=Preferred Term; SOC=System Organ Class
a: Cases may contain more than 1 PT.

3.1.2.1.2. Case Demographics

Of the 1269 cases, the ages of the patients were reported in 480 cases. The mean age was 52.1 year, median was 52 years, and the range was 14 years to 79 years.

The patient demographics by gender and age, and the country of origin of the cases retrieved are provided in Table 9. Females accounted for 99.69% (1265/1269) of the 1269 cases, while males accounted for 0.3% (4/1269). Cases were received from 3 countries. The leading reporting country for cases pertaining to this topic was the US accounting for 99.8% (1267/1269) of the 1269 cases.

Table 9: Patient Demographics of Cases Reporting PTs Related to SOC Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps) (n=1269)

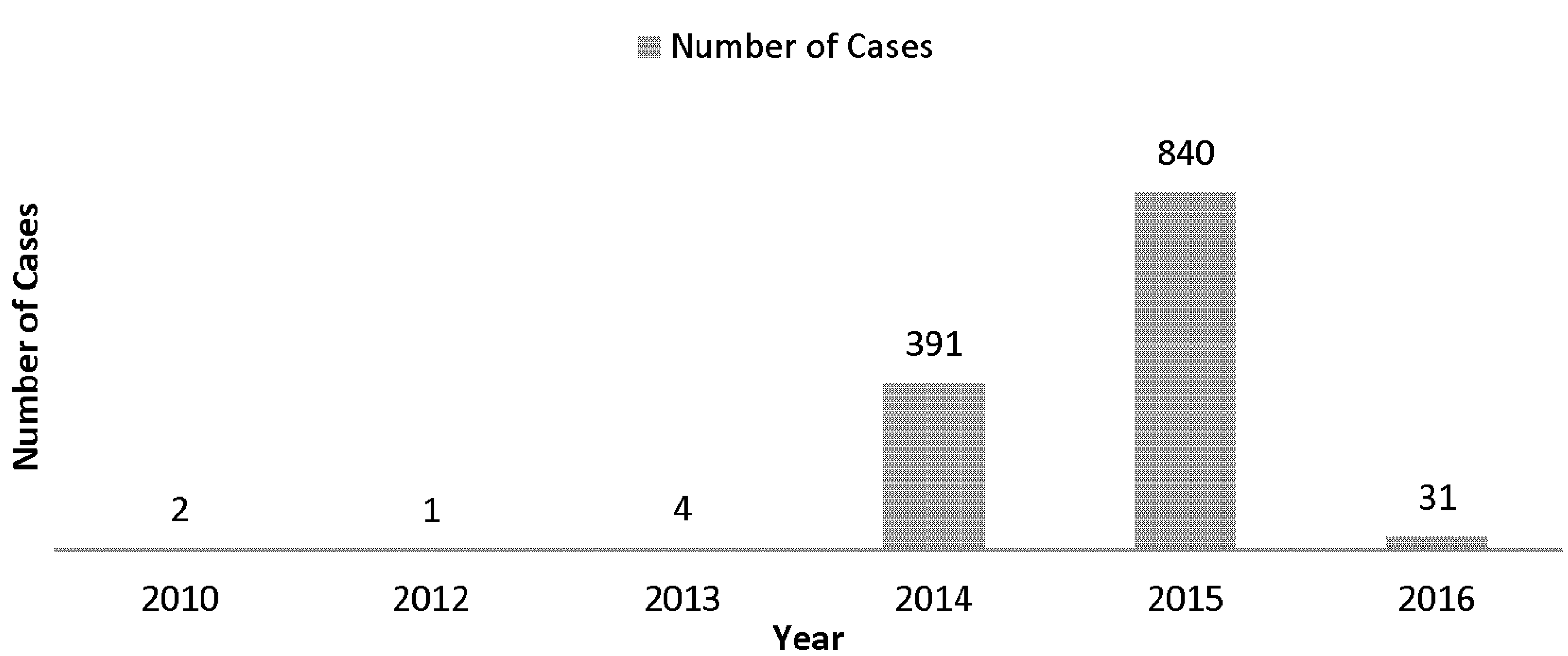
Characteristic		Number of Cases (%)
Gender	Female	1265 (99.7)
	Male	4 (0.3)
Patient Age (years)	0 to <2	0 (0)
	2 to <12	0 (0)
Mean: 52.1	12 to <18	1 (0.1)
Median: 52	18 to <35	24 (1.9)
Range: 14 to 79	35 to <50	165 (13)
	50 to <65	232 (18.3)
	≥65	58 (4.6)
	Not reported	789 (62.2)
Country of Origin	United States	1267 (99.8)
	Canada	1 (0.1)
	Germany	1 (0.1)

Key: n=Number of Cases; PT=Preferred Term; SOC=System Organ Class

3.1.2.1.3. Trending of Cases Representing SOC Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps)

Figure 3 represents the number of cases reporting PTs applicable to the Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps) SOC. The highest numbers of cases were received in the year 2015.

Figure 3: Trend Analysis of Cases Reporting PTs Related to SOC Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps)*



*Data cut-off date is 23 February 2016

3.1.2.1.4. Ovarian Cancers

A total of 1242 cases involving ovarian cancers were identified. These cases reported a PT coded to the high level term (HLT) Ovarian neoplasms malignant (excl germ cell). Of these 1242 cases, 398 were medically confirmed and 844 medically unconfirmed. Cases were received from 3 countries. The leading reporting country for these cases was the US accounting for 99.83% (1240/1242) of the cases.

The distribution of the 1242 cases (reported 1243 PTs) by stage and histological types of the ovarian cancers is presented in Table 10.

Table 10: Distribution of Ovarian Cancers by Stage and Histological Type (n=1242)

Characteristic		Number of Cases
Stage	I	68
	II	40
	III	140
	IV	43
	Not reported	952
Histological type	Serous	42
	Endometrioid	13
	Mucinous	4
	Clear cell	2
	Not reported	1182

Key: n=Number of Cases

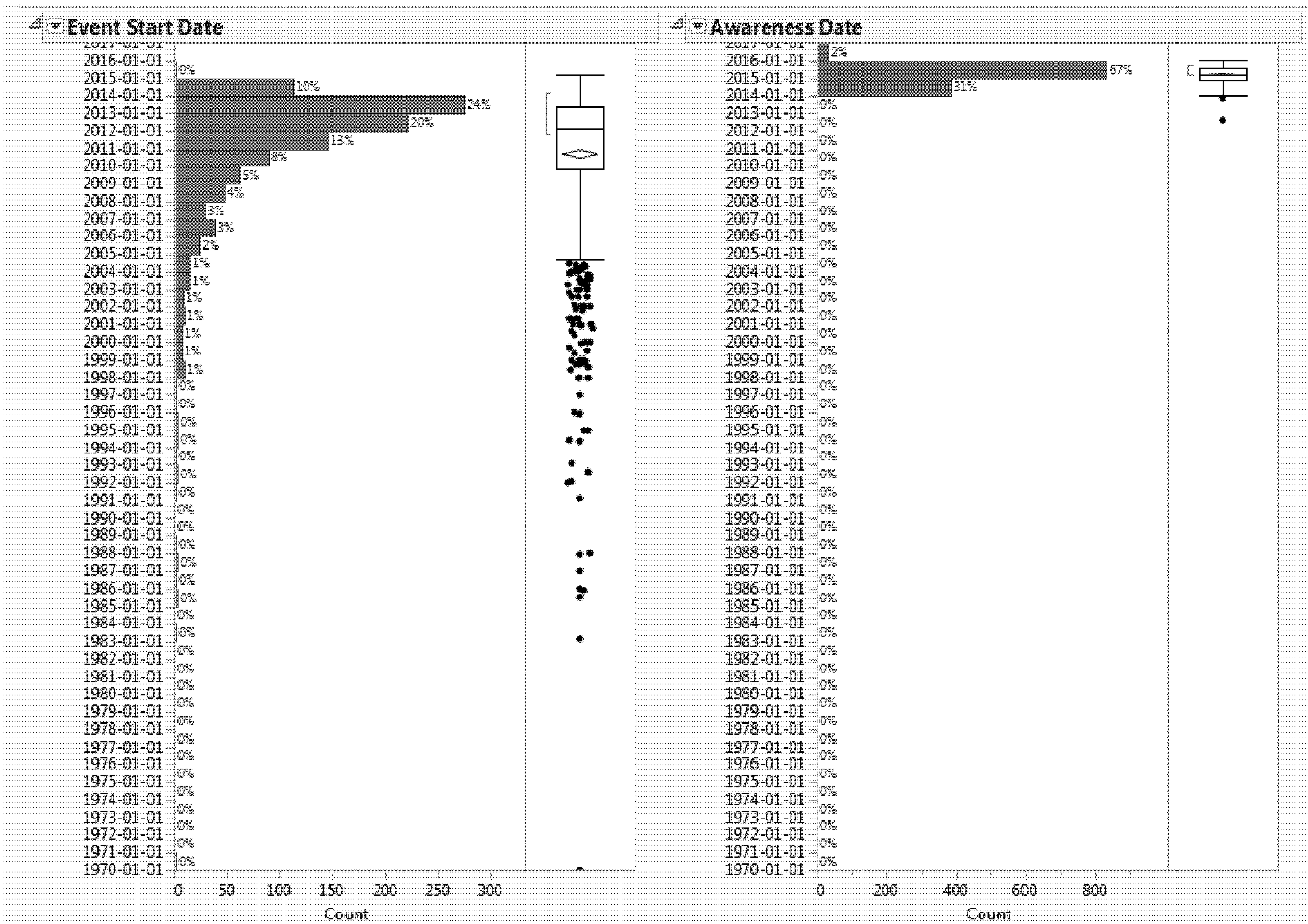
Of the 1242 cases involving ovarian cancer, 1133 cases reported the event start date and 1242 reported the Company awareness date. The mean and the median dates are presented in Table 11.

Table 11: Summary of Event Start Date and Company Awareness Date of Cases Reporting Ovarian Cancers (n=1242)

	Event Start Date	Company Awareness Date
N	1133	1242
N Missing	115	0
Mean	2010-10-02	2015-03-27
Median	2012-03-01	2015-03-23
Min	1970-01-01	2012-08-17
Max	2015-03-01	2016-02-05

Majority of the awareness dates fell between 2014 and 2015, indicating stimulated reporting (see Figure 4).

Figure 4: Reported Event Start Dates and Company Awareness Dates (1-Year Interval) of Cases Reporting Ovarian Cancers (n=1242)

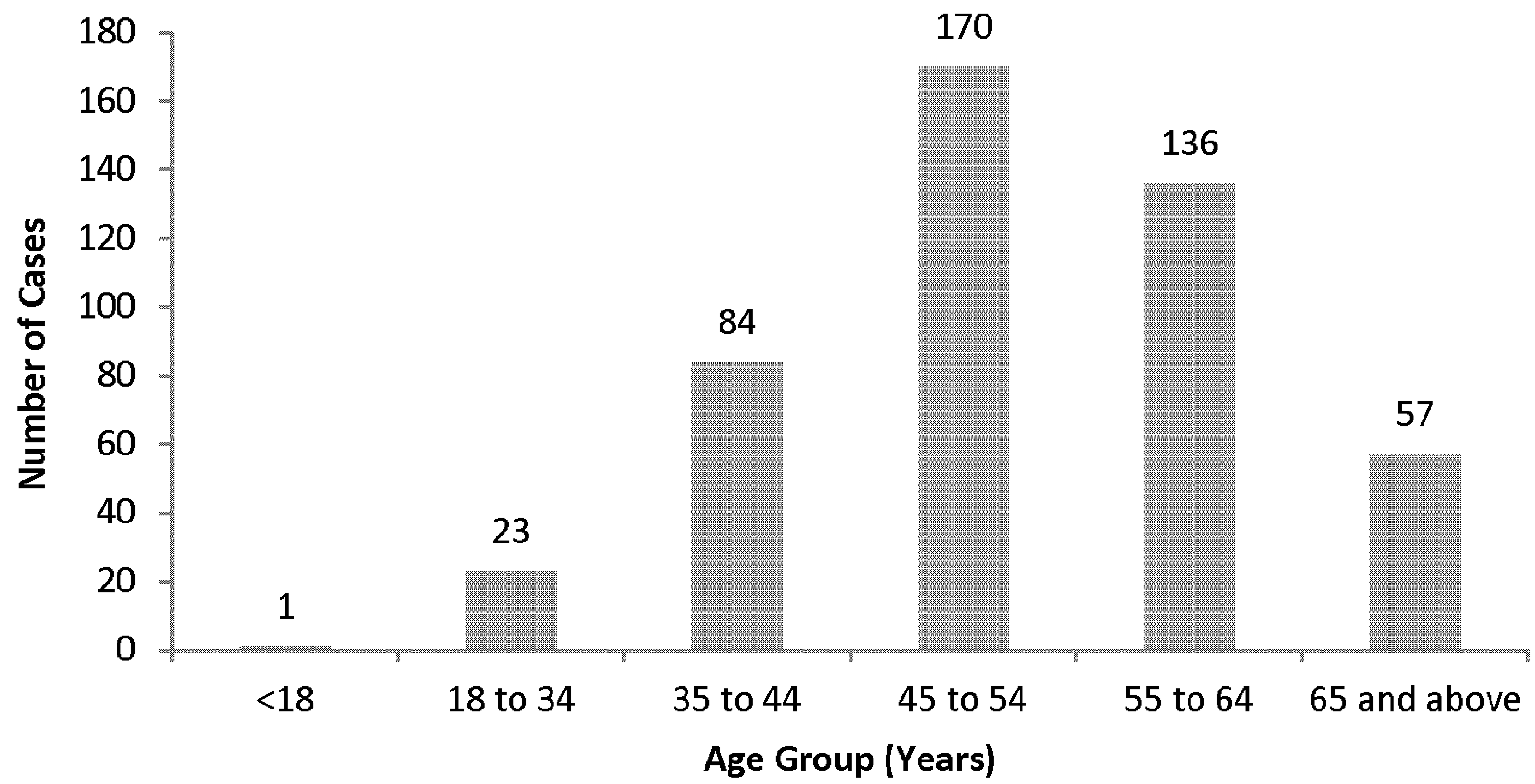


For the explanation of the legend for the plots in the above figure 4 see Attachment 2

3.1.2.1.4.1. Trending of Cases Representing Ovarian Cancers

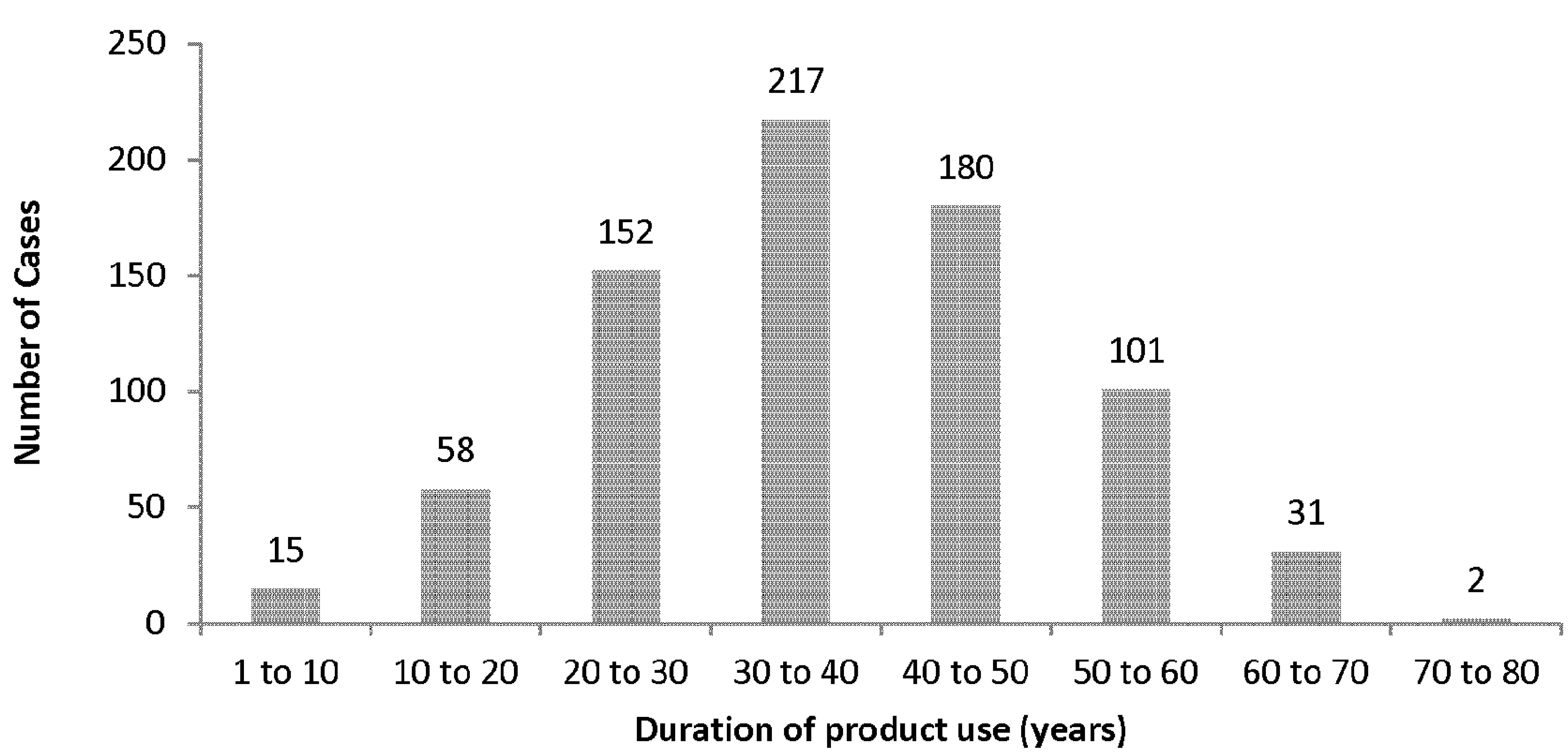
Figure 5 represents the age-wise trending of cases reporting ovarian cancers. The highest number of cases was seen in the age group 45 to 54 years, followed by the age group 55 to 64 years.

Figure 5: Age-wise Trend Analysis of Cases Reporting Ovarian Cancers (n=471)



Information on duration of product use was reported in 756 cases. Figure 6 represents the trending of cases according to the duration of use of the product. The highest number of cases (n=217) was observed with the duration of 30 to 40 years.

Figure 6: Trend Analysis of Ovarian Cancer Cases According to Duration of Product Use (n=756)



3.1.2.1.4.2. Analysis of Risk Factors of Ovarian Cancer

The 1242 cases were analysed for the presence of risk factors known to be associated with ovarian cancer. These risk factors accounted for 372 cases ie, 30% of the total cases (1242)

received for ovarian cancers. Table 12 below presents a summary of cases according to these risk factors.

Table 12: Summary of Risk Factors Associated With Cases Reporting Ovarian Cancer (n=372)

Risk Factors[a]	Number of Cases
Age	
45 years and above	363
Cancer History[b]	
Family history of cancer	25
Previous cancer	3
Medical Conditions	
Smoking/Tobacco	10
Diabetes	5
Obesity	5
Infertility	3

Key: n=Number of Cases
a: A single case may include more than 1 risk factor.
b: Includes history of ovarian and/or other cancers.

In 870 cases involving ovarian cancers, insufficient information was provided regarding the patients' age, medical conditions and reproductive history.

In the majority of cases (97.1% [1206/1242]) cases, females used the product to dust the perineal area for hygiene purposes; in the remaining cases insufficient information was provided regarding the nature of product use.

3.1.2.1.4.3. Analysis of Outcomes in Cases Involving Ovarian Cancers

Table 13 below describes the frequency of cases according to their outcomes. In the majority of cases (86% [1072/1242]) the outcomes were not reported.

Table 13: Summary of Outcomes Reported in Cases Reporting Ovarian Cancers (n=1242)

Outcome	Number of Cases
Fatal	50
Not Recovered	117
Recovered/Recovering	3
Not reported	1072
Total Number of Cases	**1242**

Key: n=Number of Cases

3.1.2.1.5. Female Reproductive Tract Cancers

A total of 46 cases involved female reproductive tract cancers (other than ovarian) and breast cancers. The distribution of these cases according to the HLTs is presented in Table 14.

Table 14: Distribution of Cases Reporting Female Reproductive Tract and Breast Cancers (n=46)

HLTs	Number of Cases[a]
Endometrial neoplasms malignant	16
Fallopian tube neoplasms malignant	12
Uterine neoplasms malignant NEC	7
Cervix neoplasms malignant	6
Breast and nipple neoplasms malignant	3
Female reproductive neoplasms unspecified malignancy	2
Uterine neoplasms benign	1

Key: n=Number of Cases; HLT=High Level Term; NEC=Not Elsewhere Classified
a: A case may report preferred terms related to more than 1 HLT.

Of these 46 cases, 19 cases including cervical neoplasms (2), endometrial neoplasms (9), fallopian tube neoplasms (5), and uterine neoplasms (3), also involved an ovarian cancer. These 46 cases provided insufficient information regarding medical history including family and reproductive history, for a meaningful medical assessment.

3.1.2.1.6. Other Cancers

A total of 29 cases involving cancers other than female reproductive tract cancers were identified. The distribution of these cases according to the HLTs is presented in Table 15.

Table 15: Distribution of Cases Reporting Other Cancers (n=29)

HLTs	Number of Cases
Neoplasms malignant site unspecified NEC	5
Gastrointestinal neoplasms malignant NEC	4
Skin neoplasms malignant and unspecified (excl melanoma)	3
Mesotheliomas malignant and unspecified	3
Metastases to specified sites	2
Carcinoid tumours	1
Central nervous system neoplasms malignant NEC	1
Colorectal neoplasms malignant	1
Gastrointestinal neoplasms malignancy unspecified NEC	1
Laryngeal neoplasms malignant	1
Leiomyosarcomas	1
Neoplasms unspecified malignancy and site unspecified NEC	1
Non-Hodgkin's lymphomas NEC	1
Pancreatic neoplasms malignant (excl islet cell and carcinoid)	1
Prostatic neoplasms malignant	1
Respiratory tract and pleural neoplasms malignant cell type unspecified NEC	1
Skin melanomas (excl ocular)	1

Key: n=Number of Cases; HLT=High Level Term; NEC=Not Elsewhere Classified

Of these 29 cases, 11 cases also involved an ovarian cancer, 6 cases involved a neoplasm of unspecified nature; 3 cases reported environmental exposure to asbestos or potentially asbestos-containing material, 2 cases reported previous cancer. The remaining 7 cases provided insufficient information on the patient's medical history for an adequate assessment.

3.1.2.2. Shower to Shower® Powder

Shower to Shower® products were divested by the Company in 2013. After the divestiture, cases reporting the use of Shower to Shower® Powders were assigned in the RSS Global Safety Database to Johnson's® Baby Powder as Company suspect or co-suspect products. The 1242 cases for ovarian cancer reviewed above include Shower to Shower Powder cases.

Overall, above review of ovarian cancer cases reported for Johnson's® Baby Powder or Shower to Shower® Powder products show that almost all cases are from legal sources. The time trend of case report as compared to the identification of the event indicates stimulated reporting. Case series review did not identify evidence of causal association.

3.1.3. SOC Injury, Poisoning and Procedural Complications

3.1.3.1. Johnson's® Baby Powder

3.1.3.1.1. Case Screening Results

The search of the RSS Global Safety Database retrieved 770 total cases reporting the use of Johnson's® Baby Powder for PTs applicable to the Injury, Poisoning and Procedural Complications SOC. The distribution of the PTs is presented in Table 16.

Table 16: Preferred Terms in Cases Concerning Johnson's® Baby Powder Representing SOC Injury, Poisoning and Procedural Complications (n=770)

MedDRA PTs	Number of PTs[a] (%)
Off label use	250 (32.5)
Accidental exposure to product by child	234 (30.4)
Accidental exposure to product	146 (19.0)
Drug administered at inappropriate site	31 (4.0)
Intentional product misuse	25 (3.2)
Wrong technique in product usage process	18 (2.3)
Exposure via ingestion	16 (2.1)
Exposure via inhalation	13 (1.7)
Laceration	13 (1.7)
Expired product administered	12 (1.7)
Exposure during pregnancy	9 (1.2)
Incorrect route of drug administration	5 (0.6)
Nail injury	5 (0.6)
Product use issue	5 (0.6)
Intentional product use issue	4 (0.5)
Injury	3 (0.4)
Maternal exposure during pregnancy	3 (0.4)
Bronchitis chemical	1 (0.1)
Exposure via direct contact	1 (0.1)
Limb injury	1 (0.1)
Occupational exposure to product	1 (0.1)
Pneumoconiosis	1 (0.1)
Poisoning	1 (0.1)
Product package associated injury	1 (0.1)

Table 16: Preferred Terms in Cases Concerning Johnson's® Baby Powder Representing SOC Injury, Poisoning and Procedural Complications (n=770)

MedDRA PTs	Number of PTs[a] (%)
Scratch	1 (0.1)
Wrong drug administered	1 (0.1)

Key: MedDRA=Medical Dictionary for Regulatory Activities; n=Number of Cases; PT=Preferred Term; SOC=System Organ Class
a: Cases may contain more than 1 PT.

3.1.3.1.2. Case Demographics

Of the 770 cases, the ages of the patients were reported in 126 cases. The mean age was 15.4 years, median was 1.5 years, and the range was 2 weeks to 85 years.

The patient demographics by gender and age, and the country of origin of the cases retrieved are provided in Table 17. Females accounted for 52.7% (406/770) of the 770 cases, while males accounted for 33.2% (256/770). The gender was not reported in 14.0% (108/770) of the cases. Cases were received from 11 countries. The leading reporting country for cases pertaining to this topic was the US accounting for 86.4% (665/770) of the 770 cases.

Table 17: Patient Demographics of Cases Concerning Johnson's® Baby Powder Representing SOC Injury, Poisoning and Procedural Complications (n=770)

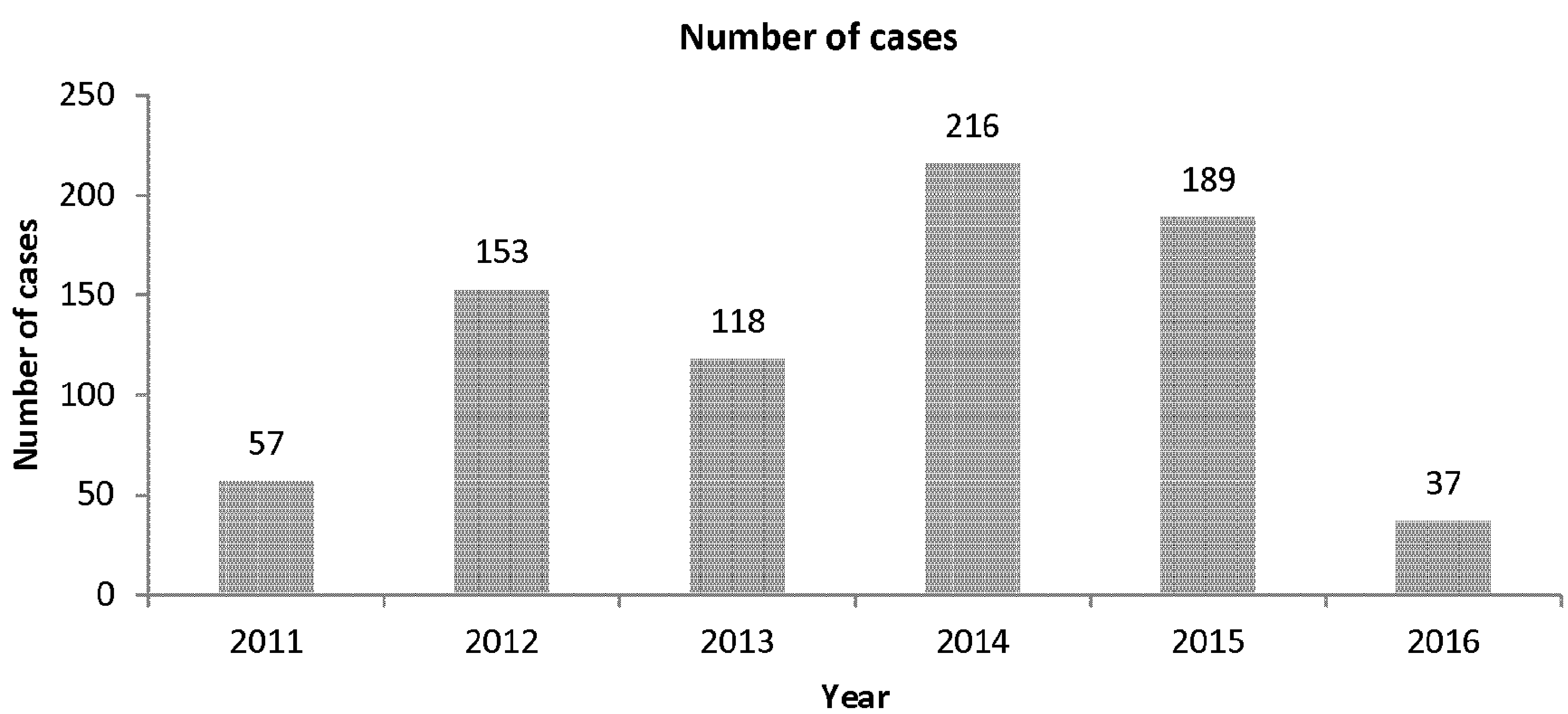
Characteristic		Number of Cases (%)
Gender	Female	406 (52.7)
	Male	256 (33.2)
	Not reported	108 (14.0)
Patient Age (years)	0 to <2	70 (9.1)
	2 to <12	21 (2.7)
Mean: 15.4	12 to <18	3 (0.4)
Median: 1.5	18 to <35	8 (1.0)
Range: 2 weeks to	35 to <50	2 (0.3)
85 years	50 to <65	12 (1. 6)
	≥65	10 (1.3)
	Not reported	644 (83.6)
Country of Origin	United States	665 (86.4)
	China	50 (6.5)
	Canada	27 (3.5)
	Australia	9 (1.2)
	Viet Nam	7 (0.9)
	Japan	4 (0.5)
	Indonesia	2 (0.3)
	Taiwan	2 (0.3)
	Thailand	2 (0.3)
	India	1 (0.1)
	New Zealand	1 (0.1)

Key: n=Number of Cases; SOC=System Organ Class

3.1.3.1.3. Trending of Cases Representing SOC Injury, Poisoning and Procedural Complications

Figure 7 represents the number of cases reporting the use of Johnson's® Baby Powder for PTs applicable to the Injury, Poisoning and Procedural Complications SOC. The highest number of cases was received in the year 2014.

Figure 7: Trend Analysis of Cases Concerning Johnson's® Baby Powder Representing SOC Injury, Poisoning and Procedural Complications for Baby powder*



*Data cut-off date is 23 February 2016

3.1.3.1.4. Case Characteristics

The following characteristics of the 770 cases were analysed and tabulated: seriousness, medical confirmation, and outcome.

Table 18 below describes the case criteria break down of the 770 cases into case level seriousness, medical confirmation, and case outcome. The majority of cases (99.1% [756/770]) were categorised as medically unconfirmed.

Table 18: Seriousness, Medical Confirmation, and Outcome of Cases Concerning Johnson's® Baby Powder Representing SOC Injury, Poisoning and Procedural Complications (n=770)

Characteristic	Number of Cases (%)
Seriousness	
Nonserious	756 (98.2)
Serious	14 (1.8)
Medically Confirmed	
No	763 (99.1)
Yes	7 (0.9)

Table 18: Seriousness, Medical Confirmation, and Outcome of Cases Concerning Johnson's® Baby Powder Representing SOC Injury, Poisoning and Procedural Complications (n=770)

Characteristic	Number of Cases (%)
Outcome	
Recovered	70 (9.1)
Not recovered	55 (7.1)
Recovering	20 (2.6)
Event ongoing	1 (0.1)
Not reported	624 (81.0)
Total Number of Cases	**770**

Key: n=Number of Cases; SOC=System Organ Class

Of the 14 serious cases, 2 cases reported hospitalization; the patients had either not recovered from the events or the outcome of the event was not reported.

Table 19 provides an overview of the SAEs reported in 14 cases.

Table 19: Serious Adverse Events in Cases Concerning Johnson's® Baby Powder Representing SOC Injury, Poisoning and Procedural Complications (n=14)

MedDRA PTs	Number of PTs[a] (%)
Accidental exposure to product	5 (35.7)
Exposure during pregnancy	2 (14.3)
Maternal exposure during pregnancy	2 (14.3)
Wrong technique in product usage process	2 (14.3)
Bronchitis chemical	1 (7.1)
Exposure via inhalation	1 (7.1)
Occupational exposure to product	1 (7.1)
Pneumoconiosis	1 (7.1)

Key: MedDRA=Medical Dictionary for Regulatory Activities; n=Number of Cases; PT=Preferred Term; SOC=System Organ Class
a: Cases may contain more than 1 PT.

Of the 14 serious cases, 6 reported events suggesting exposure of the product via inhalation. Five of these 6 cases involved accidental exposure, while the remaining case reported inhalation of the product in babies. Four of the 6 cases involving exposure via inhalation reported respiratory events, which included breathing difficulty (2), chemical bronchitis (1), cough (1), shortness of breath (1), and wheezing (1). The remaining 2 of the 6 cases involved accidental exposure to asbestos particles/fibers and reported the events laryngeal cancer and lung cancer. The cases reported insufficient information on the patients' occupation for further assessment.

Of the remaining 8 serious cases, 4 involved exposure of the product during pregnancy. Three of these 4 cases reported the events cervical cancer, ovarian cancer, and ovarian cyst; however, the cases reported insufficient information on the patients' medical or obstetric history for an adequate medical assessment. The remaining case involving exposure during pregnancy reported skin reactions and suggested a possible allergic reaction.

Of the remaining 4 serious cases, 2 involved wrong technique in product usage wherein the patient was applying baby powder with an unspecified color powder. The case reported the event

recurring facial cancer; the medical assessment of the case was confounded by the patient's previous history of cancer.

Of the remaining 2 serious cases, 1 case involved occupational exposure to product. The case reported the event malignant pleural mesothelioma. The occupation of the patient involved exposure to multiple materials containing asbestos, which confounded the medical assessment of the case. The remaining serious case reported the event talc pneumoconiosis, which is generally caused by exposure to talc dust, usually during talc mining or milling. The case reported insufficient information on the amount of talc exposure for an adequate medical assessment.

Overall, based on the review of the cases no new safety concerns were identified.

3.1.3.2. Shower to Shower® Powder

3.1.3.2.1. Case Screening Results

The search of the RSS Global Safety Database retrieved 10 total cases reporting the use of Shower to Shower® Powder for PTs applicable to the Injury, Poisoning and Procedural Complications SOC. The distribution of the PTs is presented in Table 20.

Table 20: Preferred Terms in Cases Concerning Shower to Shower® Powder Representing SOC Injury, Poisoning and Procedural Complications (n=10)

MedDRA PTs	Number of PTs[a] (%)
Accidental exposure to product	4 (40)
Intentional product misuse	3 (30)
Off label use	3 (30)
Exposure during pregnancy	1 (10)

Key: MedDRA=Medical Dictionary for Regulatory Activities; n=Number of Cases; PT=Preferred Term; SOC=System Organ Class
a: Cases may contain more than 1 PT.

3.1.3.2.2. Case Demographics

The ages of the patients were reported in all 10 cases. The mean age was 21.3 years, median was 19 years, and the range was 3 years to 42 years.

The patient demographics by gender and age, and the country of origin of the cases retrieved are provided in Table 21. Females accounted for 70% (7/10) of the 10 cases, while males accounted for 30% (3/10). All cases were received from the US in the year 2012.

Table 21: Patient Demographics of Cases Concerning Shower to Shower® Powder Representing SOC Injury, Poisoning and Procedural Complications (n=10)

Characteristic		Number of Cases (%)
Gender	Female	7 (70)
	Male	3 (30)

Table 21: Patient Demographics of Cases Concerning Shower to Shower® Powder Representing SOC Injury, Poisoning and Procedural Complications (n=10)

Characteristic		Number of Cases (%)
Patient Age (years)	0 to <2	0 (0)
	2 to <12	2 (20)
Mean: 21.3	12 to <18	0 (0)
Median: 19	18 to <35	3 (30)
Range: 3 to 42	35 to <50	2 (20)
	50 to <65	0 (0)
	≥65	0 (0)
	Not reported	3 (30)
Country of Origin	United States	10 (100)

Key: n=Number of Cases; SOC=System Organ Class

3.1.3.2.3. Case Characteristics

Table 22 below describes the case criteria break down of the 10 cases into case level seriousness, medical confirmation, and case outcome.

Table 22: Seriousness, Medical Confirmation, and Outcome of Cases Concerning Shower to Shower® Powder Representing SOC Injury, Poisoning and Procedural Complications (n=10)

Characteristic	Number of Cases (%)
Seriousness[a]	
Nonserious	10 (100)
Medically Confirmed	
No	10 (100)
Outcome	
Recovered	4 (40)
Not recovered	3 (30)
Not reported	3 (30)
Total Number of Cases	**10**

Key: n=Number of Cases; SOC=System Organ Class

All cases were medically unconfirmed and reported nonserious events. Overall, no new significant safety issue was identified.

3.1.4. SOC Respiratory, Thoracic and Mediastinal Disorders

3.1.4.1. Johnson's® Baby Powder

3.1.4.1.1. Case Screening Results

The search of the RSS Global Safety Database retrieved 114 total cases reporting the use of Johnson's® Baby Powder for PTs applicable to the Respiratory, Thoracic and Mediastinal Disorders SOC. The distribution of the PTs is presented in Table 23.

Table 23: Preferred Terms in Cases Concerning Johnson's® Baby Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders (n=114)

MedDRA PTs	Number of PTs[a] (%)
Cough	36 (31.6)
Dyspnoea	29 (25.4)
Sneezing	20 (17.6)
Choking	18 (15.8)
Asthma	6 (5.3)
Rhinorrhoea	5 (4.4)
Nasal discomfort	3 (2.6)
Throat irritation	3 (2.6)
Wheezing	3 (2.6)
Dry throat	2 (1.8)
Epistaxis	2 (1.8)
Respiratory disorder	2 (1.8)
Sinus disorder	2 (1.8)
Choking sensation	1 (0.9)
Hiccups	1 (0.9)
Idiopathic pulmonary fibrosis	1 (0.9)
Oropharyngeal pain	1 (0.9)
Pulmonary mass	1 (0.9)
Respiratory tract irritation	1 (0.9)
Rhinitis allergic	1 (0.9)
Sinus congestion	1 (0.9)
Throat tightness	1 (0.9)

Key: MedDRA=Medical Dictionary for Regulatory Activities; n=Number of Cases; PT=Preferred Term; SOC=System Organ Class

a: Cases may contain more than 1 PT.

3.1.4.1.2. Case Demographics

Of the 114 cases, the ages of the patients were reported in 104 cases. The mean age was 33.41 years, median was 32 years, and the range was 8 months to 85 years.

The patient demographics by gender and age, and the country of origin of the cases retrieved are provided in Table 24. Females accounted for 65.8% (75/114) of the 114 cases, while males accounted for 25.4% (29/114). The gender was not reported in 8.8% (10/114) of the cases. Cases were received from 7 countries. The leading reporting country for cases pertaining to this topic was the US accounting for 87.7% (100/114) of the 114 cases. The second leading reporting country was Canada accounting for 5.3% (6/114) of the cases.

Table 24: Patient Demographics of Cases Concerning Johnson's® Baby Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders (n=114)

Characteristic		Number of Cases (%)
Gender	Female	75 (65.8)
	Male	29 (25.4)
	Not reported	10 (8.8)

Table 24: Patient Demographics of Cases Concerning Johnson's® Baby Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders (n=114)

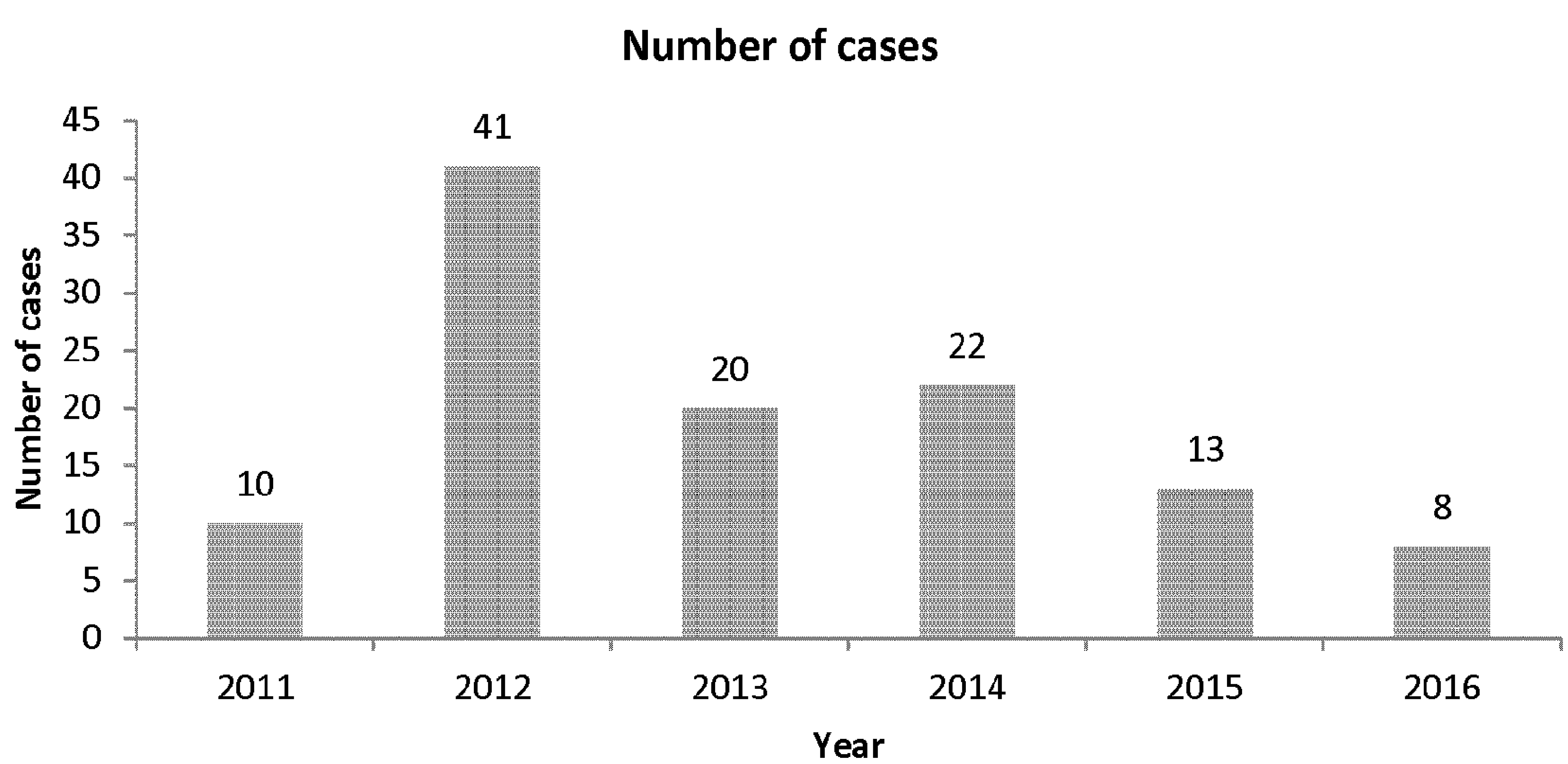
Characteristic		Number of Cases (%)
Patient Age (years)	0 to <2	11 (9.7)
	2 to <12	2 (1.8)
Mean: 33.41	12 to <18	0 (0)
Median: 32	18 to <35	4 (3.5)
Range: 8 Months to	35 to <50	0 (0)
85 years	50 to <65	7 (6.1)
	≥65	7 (6.1)
	Not reported	83 (72.8)
Country of Origin	United States	100 (87.7)
	Canada	6 (5.3)
	New Zealand	3 (2.6)
	India	2 (1.8)
	Japan	1 (0.9)
	Australia	1 (0.9)
	China	1 (0.9)

Key: n=Number of Cases; SOC=System Organ Class

3.1.4.1.3. Trending of Cases Representing SOC Respiratory, Thoracic and Mediastinal Disorders

Figure 8 represents the number of cases reporting the use of Johnson's® Baby Powder for PTs applicable to the Respiratory, Thoracic and Mediastinal Disorders SOC. The highest number of cases was received in the year 2012.

Figure 8: Trend Analysis of Cases Concerning Johnson's® Baby Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders*



*Data cut-off date is 23 February 2016

3.1.4.1.4. Case Characteristics

The following characteristics of the 114 cases were analysed and tabulated: seriousness, medical confirmation, and outcome.

Table 25 below describes the case criteria break down of the 114 cases into case level seriousness, medical confirmation, and case outcome. The majority of cases (98.2 % [112/114]) were categorised as medically unconfirmed.

Table 25: Seriousness, Medical Confirmation, and Outcome of Cases Concerning Johnson's® Baby Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders (n=114)

Characteristic	Number of Cases (%)
Seriousness	
Serious	6 (5.3)
Nonserious	108 (94.7)
Medically Confirmed	
Yes	2 (1.8)
No	112 (98.2)
Outcome	
Recovered	42 (36.8)
Not Recovered	20 (17.6)
Recovering	2 (1.8)
Not reported	50 (43.8)
Total Number of Cases	**114**

Key: n=Number of Cases; SOC=System Organ Class

3.1.4.1.5. Case Review Results

Case narratives were individually reviewed to assess potential causal relationship of the event with the product. The following screening criteria were applied:

- Cases with insufficient information,
- Cases with confounding factors.

Table 26 provides the disposition of cases on the basis of the confounding variables identified.

Table 26: Disposition of Cases Concerning Johnson's® Baby Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders (n=114)

Case Subset	Number of Cases
All Cases	
Cases With Insufficient Information	27
Cases With Confounding Factors	84
Selected Cases for Further Review	**3**

Key: n=Number of Cases; SOC=System Organ Class

Table 27 provides a breakdown of the confounding variables.

Table 27: Breakdown of Confounding Variables in Selected Cases Concerning Johnson's® Baby Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders (n=84)

Confounding Variables	Number of Cases
Medical History[a]	
Asthma[b]	14
Allergies	7
Sensitivity to fragrance	4
Allergic rhinitis	1
Lymph node cancer	1
Other Confounding Factors	
Accidental inhalation[c]	32
Accidental ingestion	23
Accidental exposure	2

Key: n=Number of Cases; SOC=System Organ Class
a: Only relevant concurrent medical condition was considered.
b: In addition to the medical history of asthma, sensitivity to fragrances and allergies were reported in some cases.
c: Four cases with accidental inhalation additionally involved accidental ingestion.

Of the 3 cases included for further review, 1 case reported the events nose irritation, sneezing, and tearing of eyes following the use of the product. The patient reported that the *"smell of product was unfamiliar and heavy."*

The remaining 2 cases reported the events dyspnoea, wheezing, and sneezing. In both the cases, the patients developed the events immediately after exposure to the product. The use of the products was discontinued and the patients were recovering from the event. A temporal association of the event with the product and a possible allergic reaction to the product cannot be ruled out.

Overall, the review of the cases did not identify any new significant safety concern.

3.1.4.2. Shower to Shower® Powder

3.1.4.2.1. Case Screening Results

The search of the RSS Global Safety Database retrieved 6 total cases reporting the use of Shower to Shower® Powder for PTs applicable to the Respiratory, Thoracic and Mediastinal Disorders SOC. The distribution of the PTs is presented in Table 28.

Table 28: Preferred Terms in Cases Concerning Shower to Shower® Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders (n=6)

MedDRA PTs	Number of PTs[a] (%)
Sneezing	4 (66.7)
Cough	3 (50.0)
Rhinorrhoea	3 (50.0)

Table 28: Preferred Terms in Cases Concerning Shower to Shower® Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders (n=6)

MedDRA PTs	Number of PTs[a] (%)
Nasal congestion	2 (33.3)
Dyspnoea	1 (16.7)

Key: MedDRA=Medical Dictionary for Regulatory Activities; n=Number of Cases; PT=Preferred Term; SOC=System Organ Class

a: Cases may contain more than 1 PT.

3.1.4.2.2. Case Demographics

Of the 6 cases, the age of the patients was reported in 2 cases. The mean age was 61 years, median was 61 years, and the range was 50 to 72 years.

The patient demographics by gender and age, and the country of origin of the cases retrieved are provided in Table 29. Females accounted for 66.67% (4/6) of the 6 cases, while males accounted for 33.33% (2/6). All cases were received from the US in the year 2012.

Table 29: Patient Demographics of Cases Concerning Shower to Shower® Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders (n=6)

Characteristic		Number of Cases (%)
Gender	Female	4 (66.7)
	Male	2 (33.3)
Patient Age (years)	0 to <2	0 (0)
	2 to <12	0 (0)
Mean: 61	12 to <18	0 (0)
Median: 61	18 to <35	0 (0)
Range: 50 to 72 years	35 to <50	0 (0)
	50 to <65	1 (16.7)
	≥65	1 (16.7)
	Not reported	4 (66.7)
Country of Origin	United States	6 (100)

Key: n=Number of Cases; SOC=System Organ Class

3.1.4.2.3. Case Characteristics

The following characteristics of the 6 cases were analysed and tabulated: seriousness, medical confirmation, and outcome.

Table 30 below describes the case criteria break down of the 6 cases into case level seriousness, medical confirmation, and case outcome. All cases were medically unconfirmed.

Table 30: Seriousness, Medical Confirmation, and Outcome of Cases Concerning Shower to Shower® Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders (n=6)

Characteristic	Number of Cases (%)
Seriousness	
Nonserious	6 (100)

Table 30: Seriousness, Medical Confirmation, and Outcome of Cases Concerning Shower to Shower® Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders (n=6)

Characteristic	Number of Cases (%)
Medically Confirmed	
No	6 (100)
Outcome	
Recovered	2 (33.3)
Not Recovered	4 (66.7)
Total Number of Cases	**6**

Key: n=Number of Cases; SOC=System Organ Class

3.1.4.2.4. Case Review Results

Case narratives were individually reviewed to assess potential causal relationship of the events with the product. The following screening criteria were applied:

- Cases with insufficient information,
- Cases with confounding factors.

Table 31 provides the disposition of cases on the basis of the confounding variables identified.

Table 31: Disposition of Cases Concerning Shower to Shower® Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders (n=6)

Case Subset	Number of Cases
All Cases	**6**
Cases with Confounding Patient Medical History	2
Cases with Insufficient Information	1
Selected Cases for Further Review	**3**

Key: n=Number of Cases; SOC=System Organ Class

Table 32 provides a breakdown of the confounding variables.

Table 32: Breakdown of Confounding Variables in Selected Cases Concerning Shower to Shower® Powder Representing SOC Respiratory, Thoracic and Mediastinal Disorders (n=2)

Confounding Variables	Number of Cases
Medical History	
Allergic to vanilla	1
Sensitivity to fragrance	1

Key: n=Number of Cases; SOC=System Organ Class

Of the 3 cases included for further review, 2 involved accidental inhalation of the product and reported the events cough (2), rhinorrhoea (2), and sneezing (2).

The remaining case involved a female who experienced difficulty in breathing 30 minutes after the use of the product SHOWER TO SHOWER ABSORBENT BODY POWDER MORNING FRESH for preventing sweating. The patient was recovering from the event following

discontinuation of the product. A temporal association of the event with the product cannot be ruled out.

Overall, the review of the cases did not identify any new significant safety concern.

3.1.5. The Remaining System Organ Classes

3.1.5.1. Johnson's® Baby Powder

3.1.5.1.1. Case Screening Results

The search of the RSS Global Safety Database retrieved 223 total cases reporting the use of Johnson's® Baby Powder for PTs applicable to the remaining SOCs. Due to less number of cases reported for these SOCs, the data here is presented in aggregate for all the remaining SOCs. The distribution of the remaining SOCs along with their number of PTs is presented in Table 33.

Table 33: Preferred Terms in Cases Concerning Johnson's® Baby Powder Representing Remaining SOCs (n=223)

SOCs/MedDRA PTs	Number of PTs[a] (%)
Infections and Infestations	**64 (28.7)**
Furuncle	43 (19.3)
Fungal Infection	7 (3.1)
Application site infection	5 (2.2)
Fungal skin infection	3 (1.3)
Application site pustules	2 (0.9)
Ear infection	1 (0.4)
Tinea infection	1 (0.4)
Urinary tract infection	1 (0.4)
Vaginal infection	1 (0.4)
Skin and Subcutaneous Tissue Disorders	**42 (18.8)**
Acne cosmetic	10 (4.5)
Eczema	5 (2.2)
Onychoclasis	5 (2.2)
Dermatitis diaper	4 (1.8)
Miliaria	3 (1.3)
Acne	1 (0.4)
Alopecia	1 (0.4)
Blister	1 (0.4)
Blood blister	1 (0.4)
Cold sweat	1 (0.4)
Dandruff	1 (0.4)
Dermatitis	1 (0.4)
Dermatitis contact	1 (0.4)
Hair colour changes	1 (0.4)
Hair growth abnormal	1 (0.4)
Hyperhidrosis	1 (0.4)
Pruritus	1 (0.4)
Rash	1 (0.4)
Skin discolouration	1 (0.4)
Skin wrinkling	1 (0.4)
Gastrointestinal Disorders	**38 (17.0)**
Vomiting	15 (6.7)
Nausea	6 (2.7)
Retching	6 (2.7)

Table 33: Preferred Terms in Cases Concerning Johnson's® Baby Powder Representing Remaining SOCs (n=223)

SOCs/MedDRA PTs	Number of PTs[a] (%)
Diarrhoea	3 (1.3)
Abdominal discomfort	1 (0.4)
Abdominal distension	1 (0.4)
Abdominal pain	1 (0.4)
Abdominal pain upper	1 (0.4)
Anorectal discomfort	1 (0.4)
Flatulence	1 (0.4)
Infrequent bowel movements	1 (0.4)
Lip swelling	1 (0.4)
Eye Disorders	**32 (14.3)**
Ocular hyperaemia	10 (4.5)
Eye irritation	6 (2.7)
Eye pruritus	6 (2.7)
Eye swelling	4 (1.8)
Lacrimation increased	4 (1.8)
Eye discharge	1 (0.4)
Visual impairment	1 (0.4)
Immune System Disorders	**20 (9.0)**
Hypersensitivity	19 (8.5)
Perfume sensitivity	1 (0.4)
Nervous System Disorders	**13 (5.8)**
Headache	4 (1.8)
Migraine	4 (1.8)
Paraesthesia	2 (0.9)
Dysgeusia	1 (0.4)
Somnolence	1 (0.4)
Sinus headache	1 (0.4)
Reproductive System and Breast Disorders	**9 (4.0)**
Infertility female	2 (0.9)
Ovarian cyst	2 (0.9)
Endometrial cancer stage I	1 (0.4)
Endometriosis	1 (0.4)
Genital haemorrhage	1 (0.4)
Ovarian disorder	1 (0.4)
Vaginal haemorrhage	1 (0.4)
Musculoskeletal and Connective Tissue Disorders	**9 (4.0)**
Pain in extremity	8 (3.6)
Back disorder	1 (0.4)
Psychiatric Disorders	**8 (3.6)**
Eating disorder	3 (1.3)
Insomnia	2 (0.9)
Abnormal behaviour	1 (0.4)
Drug dependence	1 (0.4)
Emotional distress	1 (0.4)
Metabolism and Nutrition Disorders	**6 (2.7)**
Pica	3 (1.3)
Decreased appetite	2 (0.9)
Eating disorder	1 (0.4)
Investigations	**3 (1.3)**
Blood phosphorus increased	1 (0.4)
Weight increased	1 (0.4)
Heart rate increased	1 (0.4)

Table 33: Preferred Terms in Cases Concerning Johnson's® Baby Powder Representing Remaining SOCs (n=223)

SOCs/MedDRA PTs	Number of PTs[a] (%)
Pregnancy, Puerperium, and Perinatal Conditions	**2 (0.9)**
Stillbirth	1 (0.4)
Abortion spontaneous	1 (0.4)
Vascular Disorders	**1 (0.4)**
Hot flush	1 (0.4)
Renal and Urinary Disorders	**1 (0.4)**
Dysuria	1 (0.4)

Key: MedDRA=Medical Dictionary for Regulatory Activities; n=Number of Cases; PT=Preferred Term; SOC=System Organ Class
a: Cases may contain more than 1 PT.

3.1.5.1.2. Case Demographics

Of the 223 cases, the ages of the patients were reported in 38 cases. The mean age was 31.3 years, median was 9.5 years, and the range was 2 months to 86 years.

The patient demographics by gender and age, and the country of origin of the cases retrieved are provided in Table 34. Females accounted for 54.7% (122/223) of the 223 cases, while males accounted for 27.4% (61/223) of the 223 cases. The gender was not reported in 17.9% (40/223) of the cases. Cases were received from 9 countries. The leading reporting country for cases pertaining to this topic was the US accounting for 67.3% (150/223) of the 223 cases. The second leading reporting country was India accounting for 20.6% (46/223) of the cases.

Table 34: Patient Demographics of Cases Concerning Johnson's® Baby Powder Representing Remaining SOCs (n=223)

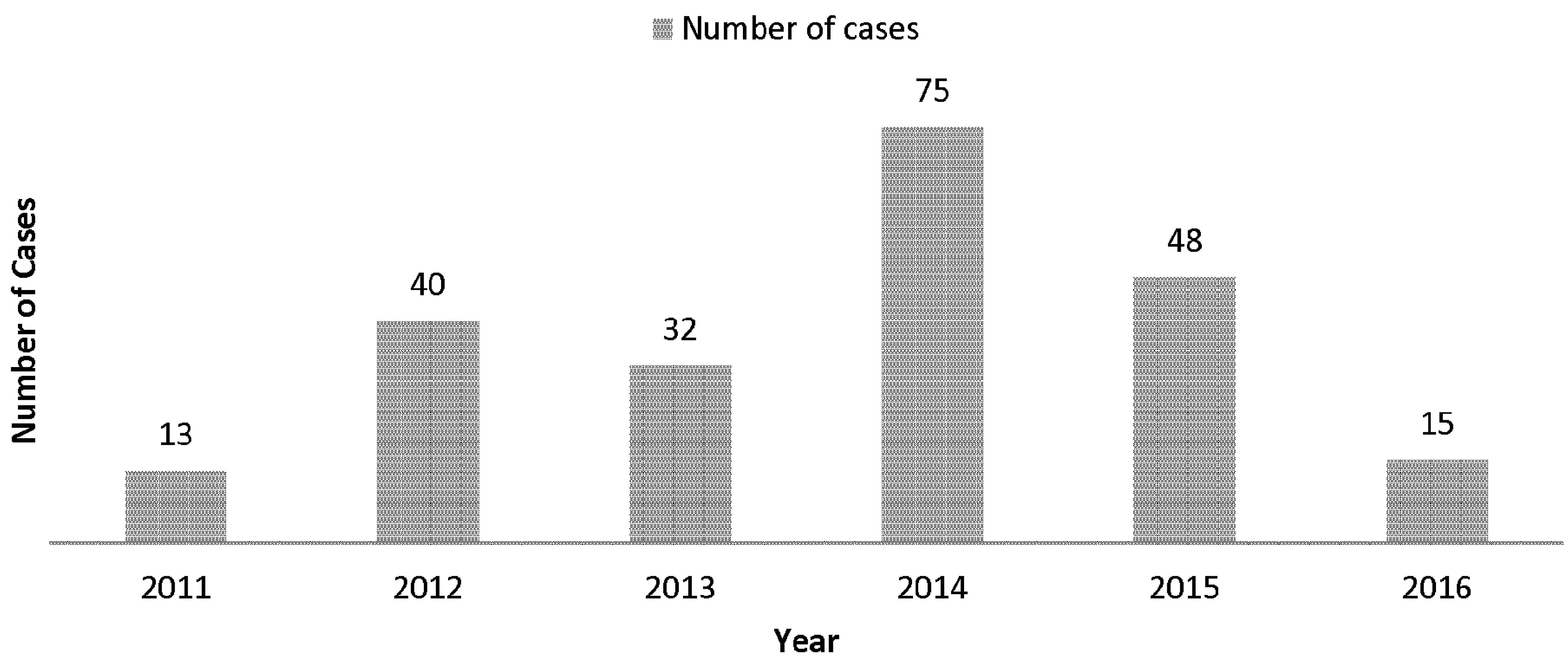
Characteristic		Number of Cases (%)
Gender	Female	122 (54.7)
	Male	61 (27.4)
	Not reported	40 (17.9)
Patient Age (years)	0 to <2	17 (7.6)
	2 to <12	2 (0.9)
Mean: 31.3	12 to <18	1 (0.4)
Median: 9.5	18 to <35	1 (0.4)
Range: 2 months to	35 to <50	2 (0.9)
86 years	50 to <65	3 (1.3)
	≥65	12 (5.4)
	Not reported	185 (83.0)
Country of Origin	United States	150 (67.3)
	India	46 (20.6)
	Canada	10 (4.5)
	China	10 (4.5)
	Australia	2 (0.9)
	Japan	2 (0.9)
	Indonesia	1 (0.4)
	Taiwan	1 (0.4)
	Viet Nam	1 (0.4)

Key: n=Number of Cases; SOC=System Organ Class

3.1.5.1.3. Trending of Cases Representing remaining SOCs

Figure 9 represents the number of cases reporting the use of Johnson's® Baby Powder for PTs applicable to the remaining SOCs. The highest number of cases was received in the year 2014.

Figure 9: Trend Analysis of Cases Concerning Johnson's® Baby Powder Representing Remaining System Organ Classes*



*Data cut-off date is 23 February 2016

3.1.5.1.4. Case Characteristics

The following characteristics of the 223 cases were analysed and tabulated: seriousness, medical confirmation, and outcome.

Table 35 below describes the case criteria break down of the 223 cases into case level seriousness, medical confirmation, and case outcome. The majority of cases (98.2% [219/223]) were categorised as medically unconfirmed.

Table 35: Seriousness, Medical Confirmation, and Outcome of Cases Concerning Johnson's® Baby Powder Representing Remaining SOCs (n=223)

Characteristics	Number of Cases (%)
Seriousness	
Serious	13 (5.8)
Nonserious	210 (94.2)
Medically Confirmed	
Yes	4 (1.8)
No	219 (98.2)

Table 35: Seriousness, Medical Confirmation, and Outcome of Cases Concerning Johnson's® Baby Powder Representing Remaining SOCs (n=223)

Characteristics	Number of Cases (%)
Outcome	
Recovered	50 (22.4)
Not Recovered	41 (18.4)
Recovering	14 (6.3)
Event Ongoing	1 (0.4)
Not reported	117 (52.5)
Total Number of Cases	**223**

Key: n=Number of Cases; SOC=System Organ Class

Of the 13 serious cases, 5 involved hospitalization. In 2 of these 5 cases, the patients were either recovering or had recovered from the events. The remaining 3 of the 5 cases reported insufficient information on the outcome for further assessment.

Most of the serious cases (69.2% [9/13]) reported limited information concerning the course of the events, patients' medical history, latency, and/or hospitalization for an adequate medical assessment. The events reported in these 9 cases reporting limited information are presented in Table 36.

Table 36: Serious Adverse Events in Cases Concerning Johnson's® Baby Powder Representing the Remaining SOCs and Reporting Limited Information (n=9)

MedDRA PTs	Number of PTs[a] (%)
Infertility female	2 (22.2)
Ovarian cyst	2 (22.2)
Abortion spontaneous	1 (11.1)
Cold sweat	1 (11.1)
Endometrial cancer stage I	1 (11.1)
Endometriosis	1 (11.1)
Hot flush	1 (11.1)
Ovarian disorder	1 (11.1)
Stillbirth	1 (11.1)

Key: MedDRA=Medical Dictionary for Regulatory Activities; n=Number of Cases; PT=Preferred Term; SOC=System Organ Class
a: Cases may contain more than 1 PT.

Of the remaining 4 cases, 1 case involved hypersensitivity to the product.

The remaining 3 cases presented with confounding variables, which included patient's age, concurrent medical condition, or surgical procedure. The events reported in these 3 cases included Eating disorder (2), Back disorder (1), and Vomiting (1).

Overall, based on the review of the cases no new safety concerns were identified.

3.1.5.2. Shower to Shower® Powder

3.1.5.2.1. Case Screening Results

The search of the RSS Global Safety Database retrieved 6 total cases reporting the use of Shower to Shower® Powder for PTs applicable to the remaining SOCs. The distribution of the PTs is presented in Table 37.

Table 37: Preferred Terms in Cases Concerning Shower to Shower® Powder Representing Remaining SOCs (n=6)

MedDRA PTs	Number of PTs[a] (%)
Eye Disorders	**3 (50.0)**
Eye pruritus	1 (16.7)
Lacrimation increased	1(16.7)
Ocular hyperaemia	1 (16.7)
Skin and Subcutaneous Tissue Disorders	**2 (33.3)**
Acne	1 (16.7)
Erythema	1 (16.7)
Immune System Disorders	**1 (16.7)**
Perfume sensitivity	1 (16.7)
Gastrointestinal Disorders	**1 (16.7)**
Nausea	1 (16.7)

Key: MedDRA=Medical Dictionary for Regulatory Activities; n=Number of Cases; PT=Preferred Term; SOC=System Organ Class
a: Cases may contain more than 1 PT.

3.1.5.2.2. Case Demographics

Of the 6 cases, the ages of the patients were reported in 05 cases. The mean age was 52.8 years, median was 64 years, and the range was 5 years to 82 years.

The patient demographics by gender and age, and the country of origin of the cases retrieved are provided in Table 38. Males accounted for 16.7% (1/16) of the cases, while females accounted for 83.3% (5/6). Cases were received from only 1 country, the US.

Table 38: Patient Demographics of Cases Concerning Shower to Shower® Powder Representing Remaining SOCs (n=6)

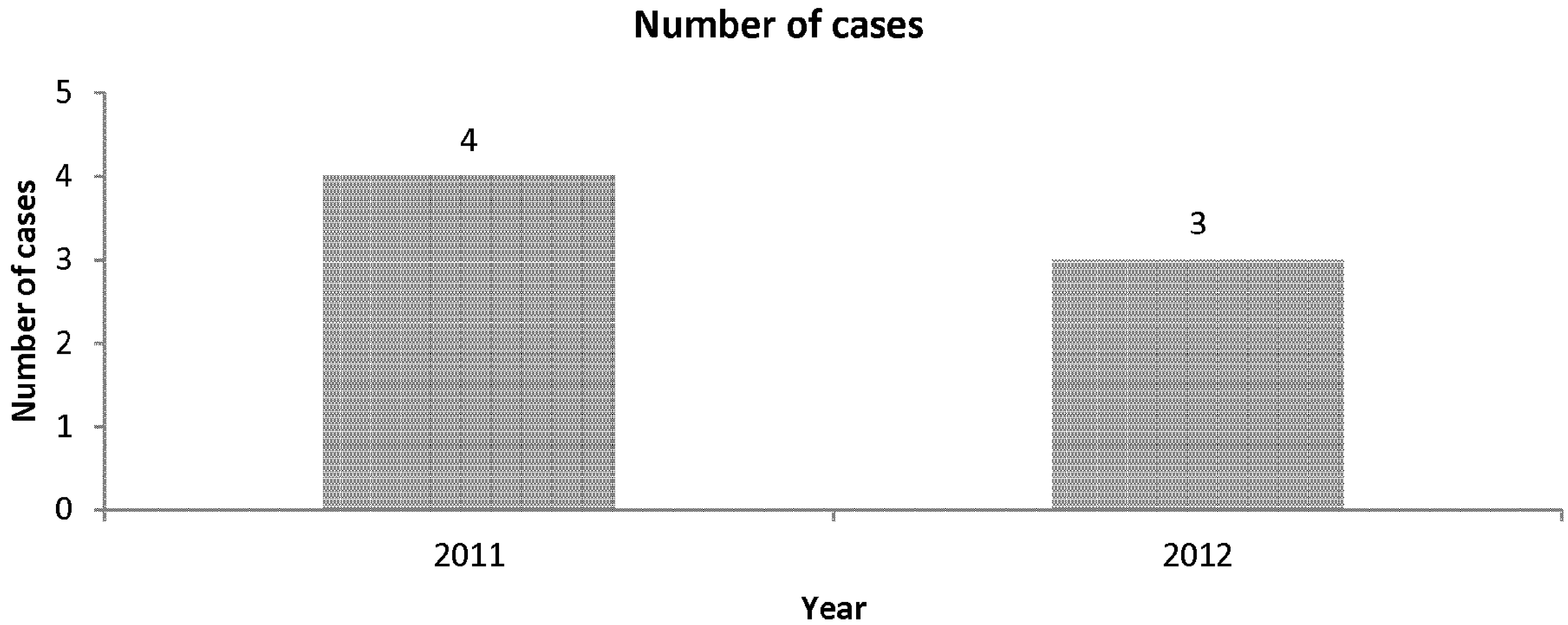
Characteristic		Number of Cases (%)
Gender	Female	5 (83.3)
	Male	1 (16.7)
Patient Age (years)	0 to <2	0 (0)
	2 to <12	1 (16.7)
Mean: 52.8	12 to <18	0 (0)
Median: 64.0	18 to <35	0 (0)
Range: 5 to 82	35 to <50	1 (16.7)
	50 to <65	1 (16.7)
	≥65	2 (33.3)
	Not reported	1 (16.7)
Country of Origin	United States	6 (100.0)

Key: n=Number of Cases; SOC=System Organ Class

3.1.5.2.3. Trending of Cases Representing remaining SOCs

Figure 10 represents the number of cases reporting the use of Shower to Shower® Powder for PTs applicable to the remaining SOCs. The highest number of cases was received in the year 2011.

Figure 10: Trend Analysis of Cases Concerning Shower to Shower® Powder Representing Remaining System Organ Classes*



*Data cut-off date is 23 February 2016

3.1.5.2.4. Case Characteristics

The following characteristics of the 6 cases were analysed and tabulated: seriousness, medical confirmation, and outcome.

Table 39 below describes the case criteria break down of the 6 cases into case level seriousness, medical confirmation, and case outcome.

Table 39: Seriousness, Medical Confirmation, and Outcome of Cases Concerning Shower to Shower® Powder Representing Remaining SOCs (n=6)

Characteristic	Number of Cases (%)
Seriousness	
Nonserious	6 (100)
Medically Confirmed	
No	6 (100)
Outcome	
Recovered	3 (50)
Not recovered	3 (50)
Total Number of Cases	**6**

Key: n=Number of Cases; System Organ Class

All cases reported non-serious events. All of the cases were categorised as medically unconfirmed. Overall, no new significant safety issue was identified.

4. SURVEILLANCE

Routine Safety Surveillance is performed as monthly and quarterly adverse event trending of safety data for Monograph Drug, Medical Device, Cosmetic, and Commodities/Consumer goods (MDC) products held in the Company's Safety Database. Safety Surveillance physicians review each case detail and make a clinical assessment of causal association between the product and event. In all of the cases the clinical assessment of causality was either unlikely or un-evaluable due to lack of information. A recent surveillance report for the time period 01 December 2015 to 31 December 2015 reported on this topic as follows.

> Increased reporting for malignant neoplasms associated with J&J BABY POWDER USA has been observed since January 2015 (and for JBABY BABY POWDER UNSPECIFIED USA since 2014), an observation possibly related with increased media awareness and legal activities surrounding the alleged issue of talc-based products and the development of cancer. As per the medical safety assessment completed by the Regional Medical Safety Officer in response to the persistent signals identified in the October 2015 report, it was stated: *"The observation in October 2015 of ovarian cancer allegedly associated with prior talc use is neither new nor changed."* It was maintained that: *"Continued monitoring of the reports of the cases is justified in that, while there exists insufficient evidence to indicate any scientific basis to any causal link between talc and cancer, additional cases could provide additional data to support or refute the allegations."* Hence, the associated signals are being assessed as *"Still for continued monitoring"*.

5. EXPOSURE

5.1. Post-Marketing Exposure

Post-Marketing Exposure

At the time of this report, only two years of shipment data for North America is available for the exposure estimation. Reporting frequencies calculated using shipment data do not reflect occurrence rates. Multiple factors influence the reporting of spontaneous experiences; therefore, caution must be exercised in the analysis and evaluation of spontaneous reports. In addition, product exposure is estimated at the time of distribution, not at the time of usage. There is a delay between the times a product is distributed until it is used by a consumer.

Consumer Exposure

The global exposure data are likely somewhat higher based on worldwide distribution and considering frequency of events based on the origin of cases. Distribution of the cases as per the country of origin in the reporting interval is presented in the Table 40.

Table 40: Distribution of the Cases Involving Johnson's Baby Powder According to the Country of Origin (01 January 2014 to 31 December 2015) (n=2,272)

Country of Origin	Number of Cases
United States of America	1,854
China	163
India	123
Canada	41
Australia	30
Viet Nam	15
Japan	12
Indonesia	7
New Zealand	7
Korea, Republic Of	5
Taiwan	4
Thailand	4
Trinidad And Tobago	3
Poland	2
Germany	1
Philippines	1

Key: n=Number of cases received in the reporting interval

Interval Exposure Estimate

Due to pragmatic reasons (current availability) only data for North America (the USA and Canada) are presented here. There were approximately 57,185,850 units of Johnson's® Baby Powder shipped by the Company in the US from 01 January 2014 to 31 December 2015 with a reporting rate of 32.42 per million units shipped (see Table 41).

Table 41: Exposure and Reporting Rate for Johnson's® Baby Powder (01 January 2014 to 31 December 2015)

Country	Number of Units	Number of cases reported	Reporting Rate (per million units shipped)
United States	57,185,850	1854	32.42
Canada	3,044,237	41	13.47
North America	60,230,087	1895	31.46

Table 43 presents the case counts per System Organ Class and Reporting Rates for Johnson's® Baby Powder.

Table 43: Case Count and Reporting Rate for Johnson's® Baby Powder by System Organ Class (01 January 2014 to 31 December 2015)

System Organ Class	Case Count	Rate (per mil. Units shipped)
Eye Disorders	4	0.07
Gastrointestinal Disorders	10	0.17
General Disorders and Administration Site Conditions	459	7.62
Immune System Disorders	8	0.13
Infections and Infestations	14	0.23
Injury, Poisoning and Procedural Complications	333	5.53
Investigations	1	0.02
Metabolism and Nutrition Disorders	2	0.03

Table 43: Case Count and Reporting Rate for Johnson's® Baby Powder by System Organ Class (01 January 2014 to 31 December 2015)

System Organ Class	Case Count	Rate (per mil. Units shipped)
Musculoskeletal and Connective Tissue Disorders	7	0.12
Neoplasms Benign, Malignant and Unspecified (incl Cysts and Polyps)	1230	*
Nervous System Disorders	2	0.03
Pregnancy, Puerperium and Perinatal Conditions	2	0.03
Psychiatric Disorders	4	0.07
Renal and urinary Disorders	1	0.02
Reproductive System and Breast Disorders	8	0.13
Respiratory, Thoracic and Mediastinal Disorders	30	0.50
Skin and Subcutaneous Tissue Disorders	8	0.13
Vascular Disorders	1	0.02

* Reporting rate not calculated due to long latency between exposure, occurrence of event and reporting dates.

The report date may be quite different from the date of occurrence of the event, thus there may not be temporal relation between the product shipment date and the event occurrence. For example, a large number of ovarian cancer cases were reported to the Company during 2014 and 2015 while their dates of event occurrence and product use were many years earlier.

6. POST-MARKETING SAFETY DATA REVIEW CONCLUSION

Post-marketing safety reports are collected from healthcare professionals, consumers, publications, clinical studies, and other sources. The reporting frequency of such reports is influenced by various factors, such as consumer awareness, clinical studies, scientific interests, media reports and legal interests. Stimulated reporting is usually seen when there are public news media reports on any particular topic of safety concern about consumer products. Increased reporting also occurs when there is a legal interest in a safety topic. Although the Johnson's® Baby Powder products have been in use for many decades, almost all cases noted above for ovarian cancer have been reported by or through attorneys in the last two years. Clinical review of these cases did not identify data to provide evidence to indicate causal association between the product use and ovarian cancer.

Inclusion of an AE as an adverse drug reaction (ADR) does not constitute an admission that medical personnel, user facility, holder of the regulatory licenses, distributor, manufacturer or product caused or contributed to a particular event. ADR determinations are not intended to be an appraisal of the medical cause of a particular event; instead, they represent an evaluation based on review of the available relevant information at the time of the evaluation according to the appropriate regulatory requirements.

ATTACHMENTS

Attachment 1: List of Johnson's® Baby Powder and Shower to Shower® Powder Company Products

Table A: List of Johnson's® Baby Powder Company Products

J&J BABY POWDER COOL CUCUMBER MELON USA
J&J BABY POWDER PURE CRNSTCH W/ALOE VITE USA
J&J BABY POWDER PURE CRNSTCH W/MAGN PTLS USA
J&J BABY POWDER USA
J&J BABY POWDER W/ LAVENDAR & CHAMOMILE USA
J&J BABY POWDER W/ VANILLA & JASMINE USA
JBABY BABY POWDER CA
JBABY BABY POWDER CORN ALOE & VIT E CA
JBABY BABY POWDER LAV & CHAMOMILE CA
JBABY BABY POWDER UNSPECIFIED CA
JBABY BABY POWDER UNSPECIFIED USA
JOHNSONS BABY MEDICATED POWDER USA
JOHNSONS BABY POWDER AP
JOHNSONS BABY POWDER CORNSTARCH AP
JOHNSONS BABY POWDER PRICKLY HEAT AP

Table B: List of Shower to Shower® Powder Company Products

STS ABS BODY POWDER ISLAND FRESH USA
STS ABS BODY POWDER MORNING FRESH USA
STS ABS BODY POWDER ORIGINAL FRESH USA
STS SPORT USA

Attachment 2: Legend for the Plots in Figure 4

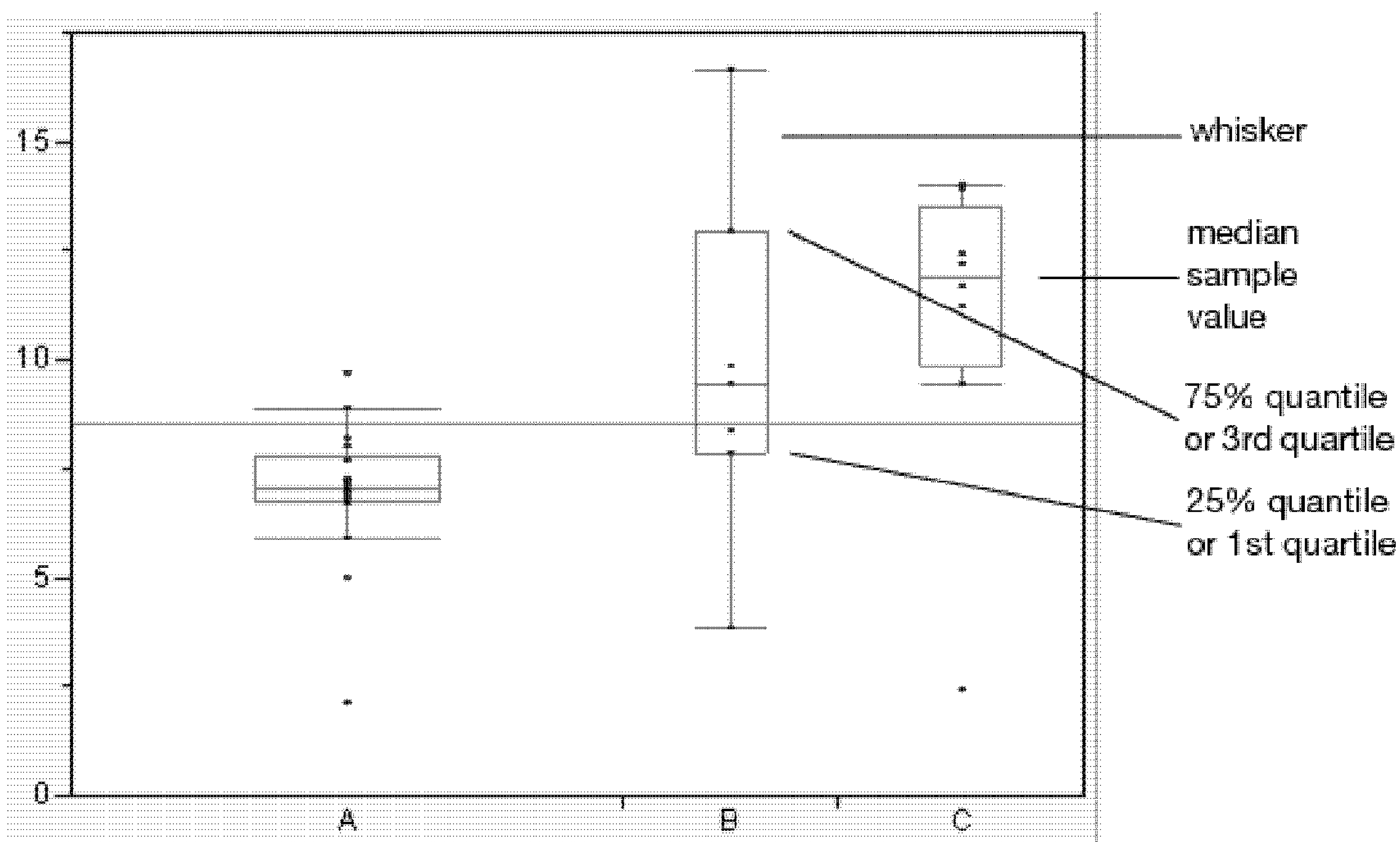
Outlier Box Plots

The outlier box plot is a graphical summary of the distribution of data. Note the following aspects about outlier box plots (see below Examples of Outlier Box Plots):

- The vertical line within the box represents the median sample value.
- The ends of the box represent the 75th and 25th quantiles, also expressed as the 3rd and 1st quartile, respectively.
- The difference between the 1st and 3rd quartiles is called the *interquartile range*.
- Each box has lines, sometimes called *whiskers*, that extend from each end. The whiskers extend from the ends of the box to the outermost data point that falls within the distances computed as follows:

```
3rd quartile + 1.5*(interquartile range)
1st quartile - 1.5*(interquartile range)
```

If the data points do not reach the computed ranges, then the whiskers are determined by the upper and lower data point values (not including outliers).



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Johnson & Johnson

Mr. Vernon Loeb
Managing Editor
Houston Chronicle

June 10, 2016

As parents caring for our children and adults taking care of ourselves and families, we are deluged by what seems like a daily avalanche of information – some true, some not, and some tainted by hidden agendas. No matter where you turn, hyperbole and misstatements often take center stage and it's difficult to know who or what to believe. At Johnson & Johnson Consumer Inc., we are guided by the medical facts and science when it comes to our products. Cosmetic talc is safe, and 30 years of scientific studies and regulatory reviews have shown this to be true.

This counters the claims of so-called experts, paid to testify on behalf of plaintiffs, who say decisions by juries should trump the overwhelming scientific data.

We first offered Johnson's Baby Powder as a product choice more than 100 years ago. Today, we continue to manufacture and sell Johnson's Baby Powder with talc because the science supports its safety.

Ovarian cancer is a devastating disease, and we recognize that women and families affected by this disease are searching for answers and want to understand the science. When concerns about an association between talc use and ovarian cancer were raised, we started doing the things you expect from a company you trust, including testing to ensure the talc in our products meets the highest quality standards, meeting with regulators and governments around the world, looking closely at the studies and available information, and talking with independent consultants.

The facts are clear. The studies, science, research and clinical evidence have continued to support the safety of cosmetic talc. Most recently, two widely-accepted, very large studies which followed women over a long period of time – the Nurses' Health Study by the Harvard School of Public Health published in 2009 and the Women's Health Initiative Observational Cohort by the U.S. National Institutes of Health published in 2014 – found no association between talc use for feminine hygiene and ovarian cancer.

There have been some studies that reported an association between talc and ovarian cancer. In my job as a scientist, terms and words matter when it comes to studies, and an 'association' does not mean something causes a specific result. Additionally, many in the scientific community have concluded that the data from those studies are inconclusive because of how the studies were conducted. Various governmental and non-governmental agencies, such as the U.S. Food and Drug Administration (FDA) and National Cancer Institute, as well as other expert panels have reviewed and analyzed the available data and concluded that there is insufficient evidence linking talc use to ovarian cancer.

Johnson's Baby Powder products contain only U.S. Pharmacopeia grade talc to ensure it meets the highest quality, purity and compliance standards. We also carefully select and process the talc used in all our global production to be asbestos-free, and have confirmed this with regular testing since the 1970s. The U.S. FDA has also independently tested and confirmed the purity of the talc used in our cosmetic products.

We trust our consumers to make their own decisions, which is why we will continue to provide consumers with the facts. As a scientist, and most importantly, as a parent, I can tell you the science is clear – cosmetic talc is, and has been, safe for use and that is the most important guiding principle for every product Johnson & Johnson Consumer Inc. offers to consumers and patients.

Tara Glasgow
Vice President, Research & Development, Global Baby
Johnson & Johnson Consumer Inc.

From: Nicholson, Susan [CPCUS]
To: Jijo James (jjames24@ITS.JNJ.com)
Sent: 7/18/2016 3:39:46 PM
Subject: FW: INFORM: Facts About Talc

Susan C. Nicholson, MD, FIDSA
Vice President Safety Surveillance and Risk Management
Consumer Products
908-745-9199 cell

Johnson & Johnson

From: Ahmed, Islah [MCCUS]
Sent: Monday, July 18, 2016 10:45 AM
To: Nicholson, Susan [CPCUS]
Subject: RE: INFORM: Facts About Talc

Hi,
As you forwarded the original message to me to provide review and input, I have reviewed and contributed to the drafts of the document in last 3 weeks and have confirmed the facts related to the medical safety aspects (against the referenced studies). Final copy is attached.
I did not see the "pictures" posted on the website before.

Kr
Islah

Islah Ahmed, MD
MSO, OCMS
Ph: 215-273-4623
Mobile: 215-264-0239

From: Nicholson, Susan [CPCUS]
Sent: Monday, July 18, 2016 10:28 AM
To: Ahmed, Islah [MCCUS]
Subject: FW: INFORM: Facts About Talc
Importance: High

Know anything about this website?

Susan C. Nicholson, MD, FIDSA
Vice President Safety Surveillance and Risk Management
Consumer Products
908-745-9199 cell

Johnson & Johnson

From: James, Jijo [CPCUS]
Sent: Monday, July 18, 2016 10:25 AM
To: Nicholson, Susan [CPCUS]
Subject: RE: INFORM: Facts About Talc

Thanks. Please let me know the moment you hear back from Islah.

From: Nicholson, Susan [CPCUS]
Sent: Monday, July 18, 2016 10:23 AM
To: James, Jijo [CPCUS]
Subject: RE: INFORM: Facts About Talc

First I am seeing, and was not invited to whatever the meeting was last Friday.

Will have a look... also, check with Islah to see if he was involved.

FYI... am meeting with Carol G. this afternoon in Skillman to plan for our Wednesday DC trip. Can check in on anything needed related to this.

Susan C. Nicholson, MD, FIDSA
Vice President Safety Surveillance and Risk Management
Consumer Products
908-745-9199 cell

Johnson & Johnson

From: James, Jijo [CPCUS]
Sent: Monday, July 18, 2016 10:21 AM
To: Nicholson, Susan [CPCUS]
Subject: FW: INFORM: Facts About Talc
Importance: High

First I am seeing this. Have we reviewed and vetted all the facts as stated?

Thanks!

From: Habib, Alexander [JJCUS]
Sent: Monday, July 18, 2016 10:04 AM
To: Whelan, Kevin [CPCUS]; Swei, Homer [CPCUS]; Reynertson, Kurt [CPCUS]; Nicholson, Susan [CPCUS]; Ahmed, Islah [MCCUS]; James, Jijo [CPCUS]; McCarthy, Timothy [CPCUS]; Ekuta, Jethro [CPCUS]; RIETZEL, MATHIAS [CONDE]; Mays, David [CPCUS]; Glasgow, Tara [CPCUS]; Carrick, Benjamin [CONGB]; Villani, Patricia [CORUS]; Van Passel, David [JPPBE]; Feldman, Jake [CORUS]; Houghton, Denise [JJCUS]; Fazio, Lil [CPCUS]; Saunders, Amy [CPCUS]; Sloan, Carrie [JJCUS]
Cc: Baer, Michele [JJCUS]; Goodrich, Carol [JJCUS]; Pound, Sandra [JJCUS]
Subject: INFORM: Facts About Talc
Importance: High

All,

Thank you again for your review of our talc microsite. This past Friday evening, Facts About Talc was officially launched.

Facts About Talc represents an important digital communication asset to be used by our company and reflects our in-depth position. Please see the link below and let Michele or I know if you have any questions.

http://www.factsabouttalc.com/

Thank you all again for your time, diligence, and attention to this project.

Alexander A. Habib | Sr. Manager, Consumer Public Affairs – North America
Johnson & Johnson Consumer, Inc. | M: 732.325.8042

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Withheld for Privilege

From: Ballman, Peggy [MCCUS]
To: Swei, Homer [CPCUS]
Sent: 12/9/2015 6:32:03 PM
Subject: FW: INPUT NEEDED: Talc website update draft
Attachments: Talc Safety Website Update with changes 12.8.15.docx

Came across this review from the UK and would like to include it in the infographic. It's largely positive to our position and it would be great to have something specific from Europe. Any concerns?

Cancer Research UK
http://www.cancerresearchuk.org/about-cancer/causes-of-cancer/cancer-controversies/cosmetics-and-toiletries#Cosmetics2

From: Swei, Homer [CPCUS]
Sent: Tuesday, December 08, 2015 2:41 PM
To: Ballman, Peggy [MCCUS]
Subject: RE: INPUT NEEDED: Talc website update draft

http://www.fda.gov/Cosmetics/ProductsIngredients/Ingredients/ucm293184.htm

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From: Ballman, Peggy [MCCUS]
Sent: Monday, December 07, 2015 2:40 PM
To: Swei, Homer [CPCUS]
Subject: INPUT NEEDED: Talc website update draft

Homer, as part of the ongoing focus on talc, our team updated the talc content on the safety website, which is attached. We sent this to our AP and LA colleagues for their feedback and because Carol wanted to ensure any updates reflected a global voice. Attached is the draft content with some changes tracked from Asia Pac. I expect to receive feedback from LA soon, and in the meantime, am sending this to you for your comments and review. If you scroll down the email chain below to the highlighted section, it explains our approach to the changes.

There's a lot to cover here, including a proposed Infographic, so it might be best for you and I to meet and discuss after you have a chance to review. What do you think?
Peggy

From: SAITZYK, Mitzi [JJPAU]
Sent: Thursday, December 03, 2015 12:27 AM
To: Ballman, Peggy [MCCUS]; Scavazzini, Ana Carolina [CONBR]
Subject: RE: INPUT NEEDED: Talc website update draft

Peggy
As promised, please find attached input from the Asia Pacific team on the proposed changes to the Talc page on Safety and Care Commitment.com

Though I have consolidated feedback, the tracked changes and comments reflect recommendations that have come from Corp Comms, Regulatory and Medical Affairs. The key for us is offering further context or information about the NTP as we would not normally refer to them in this region and they therefore lack the clout/authority that they seem to be positioned with in our statement.

I hope this is helpful to you and invite you to repeat this process in the future as additional ingredient pages go under revision. We are very happy to be consulted and welcome the opportunity to contribute.

Warm regards,
Mitzi

Mitzi Saitzyk

T: +61 2 8260 8523
M: +61 439 777 596

From: Ballman, Peggy [MCCUS]
Sent: Monday, 30 November 2015 11:32 PM
To: SAITZYK, Mitzi [JJPAU]; Scavazzini, Ana Carolina [CONBR]
Subject: RE: INPUT NEEDED: Talc website update draft

Thanks, Mitzi.

From: SAITZYK, Mitzi [JJPAU]
Sent: Monday, November 30, 2015 2:03 AM
To: Ballman, Peggy [MCCUS]; Scavazzini, Ana Carolina [CONBR]
Subject: RE: INPUT NEEDED: Talc website update draft

Hi Peggy,
Just an update from me. I've made some edits in tracked changes and have passed along to our regulatory team to input their comments as well. I should have something back to you in about 48 hours.

Thank you for offering us the opportunity to review.

Regards
Mitzi

Mitzi Saitzyk

T: +61 2 8260 8523
M: +61 439 777 596

From: Ballman, Peggy [MCCUS]
Sent: Wednesday, 25 November 2015 2:33 AM
To: Scavazzini, Ana Carolina [CONBR]; SAITZYK, Mitzi [JJPAU]
Subject: RE: INPUT NEEDED: Talc website update draft

Hi Carol, and thanks for following up. I was going to ask you and Mitzi (copied here) to do the following: Yes, please review the document from the perspective of your markets/regions and offer any thoughts or comments. In the email chain below, I've highlighted below our key objectives in updating the talc content and it would be helpful to keep those in mind as you look at the copy.

Importantly, we are always hoping to cite non-U.S. regs/studies/sources to support the safety story from a global perspective, as Carol noted below. However, I worked with Homer Swei on that very question and there were very few ex-U.S. studies, etc. available, and what we found are included in the document. That said, your local reg people might know of other approvals or supporting reg statements from your region that could be help, so they should review this as well.

Thank you both very much! Any questions, just ask. I will be on-line thru tomorrow and then we are closed for our Thanksgiving holiday until Monday. J
Peggy

From: Scavazzini, Ana Carolina [CONBR]

Sent: Monday, November 23, 2015 8:14 AM
To: Ballman, Peggy [MCCUS]
Subject: RE: INPUT NEEDED: Talc website update draft

Hi Peggy,

How are you?

Please, let me know how can I help. Should I consider the document sent by Carol to send you the suggestions from LatAm?

Thank you,

Carol

From: Ballman, Peggy [MCCUS]
Sent: terça-feira, 17 de novembro de 2015 21:21
To: Goodrich, Carol [JJCUS]
Cc: Scavazzini, Ana Carolina [CONBR]; SAITZYK, Mitzi [JJPAU]
Subject: RE: INPUT NEEDED: Talc website update draft

Thanks, Carol. Will follow up with our team and the R&D folks.

From: Goodrich, Carol [JJCUS]
Sent: Tuesday, November 17, 2015 6:05 PM
To: Ballman, Peggy [MCCUS]
Cc: Scavazzini, Ana Carolina [CONBR]; SAITZYK, Mitzi [JJPAU]
Subject: FW: INPUT NEEDED: Talc website update draft

Peggy,

Generally speaking I support the approach. However, I think we should further globalize the infographic and include “facts/content” specific to Latin America and Asia Pacific so please reach out to Carolina and Mitzi to get their suggestions on who/what we should include. In parallel, I agree we need to get input from Homer and Lorena as well. Thanks for moving this forward.

Carol

From: Ballman, Peggy [MCCUS]
Sent: Thursday, November 12, 2015 2:26 PM
To: Goodrich, Carol [JJCUS]
Subject: FW: INPUT NEEDED: Talc website update draft

Carol, I’m resending this draft of the website revisions with the infographic now dropped into the copy. Christina and I have gone back and forth a few times on the infographic. I think it can be refined further -- shorten some of the copy and jazz up the colors – but I wanted you to see everything as it stands now and get your thoughts. Also, once approved, the infographic will accompany the TP article. Other points about the changes to the general website copy are in my email below.

If you are okay with the general tone and approach, the next step would be for me to review everything with Homer or Lorena to confirm that the phrasing on safety is accurate and confirm the references, etc. After that, it still needs Legal review. Thanks.
Peggy

From: Ballman, Peggy [MCCUS]
Sent: Tuesday, November 03, 2015 12:42 PM
To: Goodrich, Carol [JJCUS]
Subject: INPUT NEEDED: Talc website update draft

Carol , as we discussed, attached is a draft of the revision to the talc section on the Safety website.

This update is based on several discussions with Alex/Homer and the feedback from the talc team. Our primary goal was to make the language more casual to the degree possible and to be more transparent about concerns/misperceptions, such as asbestos. Also, we wanted to make the content more relatable to other stakeholders, such as retailers. Some other new elements that have been added:

- Use of a female scientist/toxicologist to better relate and connect with women searching for information
- More use of graphics or Infographics
- Use of external KOL comment/statement

Keep in mind, any changes we make are limited by the current site infrastructure. According to Alex, the current platform will not be updated until next year, so we're stuck with the basic design and layout that exists now. Also, we had hoped to use some video to liven up the content, but we can't insert any video unless it will exist in a separate external vehicle, such as YouTube. Not sure why this is, but it is. For example, all the videos now on the safety website are also housed on YouTube, which makes sense for those topics. Not sure it does for talc, but we can discuss off-line. As an alternative, we can direct people to the website's other videos, which I did at the end of the copy for people who wanted more background on our toxicology testing.

Look forward to your feedback and anyone else we want to bring in. I know you're tied next couple of days, but let me know if you have any time Thurs. or Friday to catch up. Also, below is the link to the current page. Thanks.
Peggy

http://www.safetyandcarecommitment.com/ingredient-info/other/talc

Peggy Ballman
Director, Global Issues Management
Johnson & Johnson Consumer, Inc.
908-310-7721 (mobile)
pballman@its.jnj.com

CONFIDENTIAL DRAFT FOR REVEW – 12.8.15

Safety & Care Commitment Website – Talc Tab

Talc

Baby Powder made from cosmetic talc is one of JOHNSON'S® oldest products and a longtime part of baby care rituals. JOHNSON'S® Baby Powder continues to be popular with adults as well, and in many parts of the world, it remains an essential part of makeup and skin care routines. Talc is also used in toothpaste, chewing gum, aspirin, and other consumer products. With over 100 years of use, few ingredients have the same demonstrated performance, mildness and safety profile as cosmetic talc.

Any amount of talc used in a consumer product is required to be asbestos-free and has been since the 1970s -- though misperceptions still exist that talc products contain asbestos, a substance with links to cancer. JOHNSON'S® Baby Powder products contain only U.S. Pharmacopeial (USP) grade talc which meets the highest quality, purity and compliance standards. The talc used in all our global products is carefully selected and processed to be asbestos-free, and we confirm this with regular testing. The U.S. Food and Drug Administration (FDA) has also tested and confirmed the purity of our talc.[1]

Another misperception is that talc in baby powder can be easily inhaled or absorbed into the body. We always recommend not using talc around a baby's face or mouth, and to further protect your baby, we precisely mill our JOHNSON'S talc products to a relatively large size to decrease the potential to be inhaled or absorbed into the body.

Pic here of Joan Casalvieri, Ph.D., Director of Toxicology, Skincare, Johnson & Johnson Consumer, Inc.

"As a toxicologist in our Consumer business, my job is to make certain a product is safe by ~~precisely measuring whether~~ assessing any ingredient in that product poses a risk. We want to assure women and caregivers who use our talc products that numerous studies support its safety, and these include assessments by external experts in addition to our company testing. Many research papers and epidemiology studies have specifically evaluated talc and perineal use and these studies have found talc to be safe. For example, the Nurses' Health Study (2010)[2] and the Women's Health Initiative Observational Cohort (2014)[3] are two large-scale prospective studies looking at talc and ovarian cancer. Both found no causal relationship between talc and ovarian cancer."

Decades of Safety

Our confidence in using talc reflects more than 30 years of research by independent scientists, review boards and global authorities, which have all concluded that talc can be used safely in personal care products. Various government agencies and other bodies also have examined talc to determine the potential for any safety risks, and none have concluded that there are safety risks. In fact, no regulatory agency has ever required a change in labeling to reflect any safety risk from talc powder products.

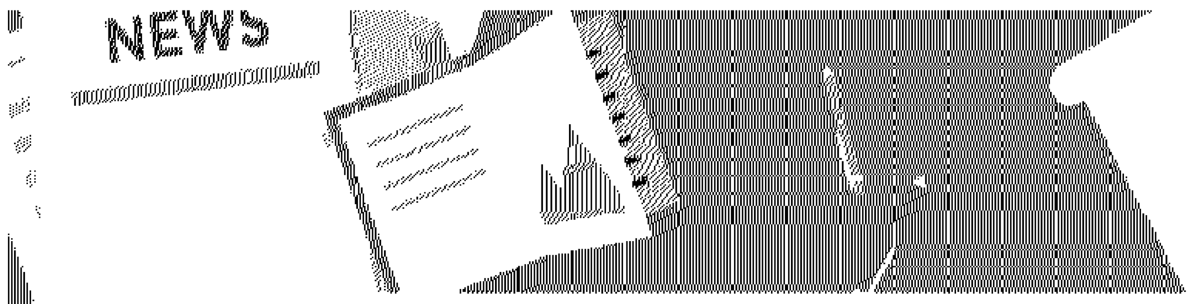
As an ingredient that is popular around the globe, many countries have approved the use of talc, among them the United States, those in the European Union, Canada, Argentina, Brazil, China, India, Israel, South Africa, Turkey, and Indonesia.

Among the agencies that have examined talc are the U.S. Department of Health and Human Services and the U.S FDA. As recently as 2014, the FDA again reviewed the safety data on talc and found "...no compelling new data or scientific evidence..." on the safety of talc.[4]

CONFIDENTIAL DRAFT FOR REVEW – 12.8.15

Cosmetic talc is not included in the most recent Report on Carcinogens, which is published by the U.S. National Toxicology Program (NTP). NTP is a globally recognized program and is formed from parts of several different government agencies, including the National Institutes of Health (NIH), the Centers for Disease Control and Prevention (CDC), and the Food and Drug Administration (FDA).

CONFIDENTIAL DRAFT FOR REVEW – 12.8.15



TALC SAFETY

A Compelling Body of Evidence

Regulators and health experts around the world have reviewed the evidence on talc, and epidemiology and other studies published over the last 30 years have assessed talc's safety. The conclusion is that cosmetic talc is a safe ingredient for personal care products.

Global Authorities	& Research Studies and Reviews
U.S. Department of Health and Human Services	European Journal of Cancer Prevention, 2007, Muscat
U.S. Food and Drug Administration	The Journal of the National Cancer Institute, Review of Nurses' Healthy Study, Gertig
National Toxicology Program	Cancer Causes & Control, 2011, data from Australian National Endometrial Cancer Study, Neill
U.S. Center for Disease Control	Cosmetic Ingredient Review (CIR), 2013
European Union	Add Here
Health Canada	Add Here

This box will read:
Health Canada and other global markets.

Note: The Houghton study will be added here.

The Cancer Research UK study will be added here.

Cosmetic Talc has been used for more than
100 years by MILLIONS of people
around the world.

CONFIDENTIAL DRAFT FOR REVEW – 12.8.15

In addition to government health authorities, our own toxicology teams are also responsible for evaluating any *new r*esearch published on talc, and at times we may ask outside experts for an independent perspective on new or existing studies. We have carefully assessed all available data on talc and consumers can feel confident that the overwhelming body of research and clinical evidence continues to support the safety of cosmetic talc.

For more about how our toxicology testing works, click here: https://youtu.be/WXvC2S0LsaI
(This links to Mona Nair video found on our Five Level Safety Assurance Process tab, Level 2).

Our Position on Talc
At Johnson & Johnson Consumer, Inc., our confidence in using talc is based on a long history of safe use and more than 30 years of research by independent researchers, scientific review boards and global regulatory authorities. Various agencies and governmental bodies have examined whether talc is a carcinogen, and none have concluded that it is. With over 100 years of use, few ingredients have the same demonstrated performance, mildness and safety profile as cosmetic talc.

References and Resources:

[1] U.S Food and Drug Administration
http://www.fda.gov/Cosmetics/ProductsIngredients/Ingredients/ucm293184.htm

[2]Gertig, Prospective Study of Talc Use and Ovarian Cancer, *Journal of the National Cancer Institute*, Nurses Health Study
http://jnci.oxfordjournals.org/content/92/3/249.full

[3]Neill, Use of talcum powder and endometrial cancer risk, Cancer Causes and Control
http://rd.springer.com/article/10.1007%2Fs10552-011-9894-5

[4] FDA Talc Letter (2).pdf – See attachment. (Note to copy reviewers: would prefer not to include a PDF of this letter as part of public references. Would that be okay?)

Petition_Denial_Letter.pdf

[5] National Toxicology Program
http://ntp.niehs.nih.gov/index.cfm?objectid=03CA6E02-FBD5-5C52-9699F9DD00863ED7

Note to Copy Reviewers: These are references for the global authorities and studies included in the infographic. They will not be noted on the website.

Global Authorities:
U.S. Department of Health and Human Services, U.S. Center for Disease Control

CONFIDENTIAL DRAFT FOR REVEW – 12.8.15

National Toxicology Program http://ntp.niehs.nih.gov/index.cfm?objectid=03CA6E02-FBD5-5C52-9699F9DD00863ED7

U.S. Food and Drug Administration
http://www.fda.gov/Cosmetics/ProductsIngredients/Ingredients/ucm293184.htm
Reg Toxicol Pharmacol pdf

National Toxicology Program
http://ntp.niehs.nih.gov/index.cfm?objectid=03CA6E02-FBD5-5C52-9699F9DD00863ED7

European Union
http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.results&annex_v1=III&search

Health Canada
http://www.hc-sc.gc.ca/cps-spc/cosmet-person/hot-list-critique/hotlist-liste-eng.php#t1

Research Studies & Reviews:

Muscat, Perineal talc use and ovarian cancer risk: a case study of scientific standards in environmental epidemiology, *European Journal of Cancer Prevention*
http://www.ncbi.nlm.nih.gov/pubmed/21712717

Gertig, Prospective Study of Talc Use and Ovarian Cancer, *Journal of the National Cancer Institute*, Nurses Health Study
http://jnci.oxfordjournals.org/content/92/3/249.full

Neil, Use of talcum powder and endometrial cancer risk, *Cancer Causes & Control,* March 2012, Volume 23, Issue 3, pp 513-519
http://rd.springer.com/article/10.1007%2Fs10552-011-9894-5

Cosmetic Ingredient Review
http://www.cir-safety.org/sites/default/files/talc032013rep.pdf#zoom=125

Houghton, Perineal powder use and risk of ovarian cancer. *JNCI J Natl Inst* 2014; 106(9).
http://jnci.oxfordjournals.org/content/106/9/dju208.full.pdf+html

Cancer Research UK
http://www.cancerresearchuk.org/about-cancer/causes-of-cancer/cancer-controversies/cosmetics-and-toiletries#Cosmetics2

Withheld for Privilege

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

APR 1 - 2014

Samuel S. Epstein, M.D.
Cancer Prevention Coalition
University of Illinois at Chicago
School of Public Health, MC 922
2121 West Taylor Street, Rm. 322
Chicago, Illinois 60612

RE: Docket Numbers 94P-0420 and FDA-2008-P-0309-0001/CP

Dear Dr. Epstein:

This letter is in response to your two Citizen Petitions dated November 17, 1994 and May 13, 2008, requesting that the Food and Drug Administration (FDA or the Agency) require a cancer warning on cosmetic talc products. Your 1994 Petition requests that all cosmetic talc bear labels with a warning such as "Talcum powder causes cancer in laboratory animals. Frequent talc application in the female genital area increases the risk of ovarian cancer." Additionally, your 2008 Petition requests that cosmetic talcum powder products bear labels with a prominent warning such as: "Frequent talc application in the female genital area is responsible for major risks of ovarian cancer." Further, both of your Petitions specifically request, pursuant to 21 CFR 10.30(h)(2), a hearing for you to present scientific evidence in support of this petition.

We have carefully considered both of your Petitions. We are committed to the protection of the public health and share your interest in reducing the risk of ovarian cancer. Current regulations state that cosmetic products shall bear a warning statement whenever necessary or appropriate to prevent a health hazard that may be associated with a product. FDA may publish a proposal to establish a regulation prescribing a warning statement on behalf of a petitioner if the petition is supported by adequate scientific basis on reasonable grounds.

After careful review and consideration of the information submitted in your Petitions, the comments received in response to the Petitions, and review of additional scientific information, this letter is to advise you that FDA is denying your Petitions. FDA did not find that the data submitted presented conclusive evidence of a causal association between talc use in the perineal area and ovarian cancer.

For this reason and for the additional reasons described below, FDA is denying your Petitions.

I. Discussion

The basis of your request, throughout both Petitions, can be summarized as comprising three major points:

1. Talc may be associated with asbestos.
2. Talc is a carcinogen based on the findings of a 1993 National Toxicology Program study.
3. Epidemiological studies confirm the causal relation between genital application of talc and ovarian cancer, and the protective effect of tubal ligation or hysterectomy, preventing the translocation of talc to the ovary.

As the points you raise in your Petitions concern the chemistry and toxicology of talc, the epidemiology associated with talc use, and the etiology of ovarian cancer, commensurate reviews were conducted to assess your request.

<u>Chemistry Findings:</u>

Asbestos is a known carcinogen and your first major point is that talc may be associated with asbestos. As evidence that talc cosmetic products contain asbestos, you first cite a 1968 survey of 22 talcum products that found fiber content averaging 19% in all 22 products. This author further concludes that "the fibrous material was predominantly talc but probably contained minor amounts of tremolite, anthophyllite, and chrysotile [asbestos-like fibers] as these are often present in fibrous talc mineral deposits …"

You then cite a follow up study from 1971-1975 that examined 21 samples of consumer talcums and powder and concluded that cosmetic grade talc was not used exclusively in these products. This study found the presence of asbestiform anthophyllite and tremolite, chrysotile, and quartz. From these two citations, one may infer that currently available talc-containing cosmetic products are presently contaminated with asbestos, a known carcinogen. Unfortunately, you did not present any original data on the chemical composition of talc currently being used in cosmetics talc products or data linking these findings to currently used talc.

It has been reported in the scientific literature that most talc products in world trade are impure as a result of the geological processes involved in the formation of talc deposits. Further, talc containing asbestos fibers such as tremolite asbestos or chrysotile are sometimes encountered. However, large deposits of high purity, asbestos-free talc do exist and talc purification techniques have been developed which can be used to improve talc quality. Thus, while it has been reported in the past that cosmetic talc has been contaminated with asbestos, it has been also reported that asbestos-free talc deposits do exist. In addition, techniques do exist for the purification of talc in order to improve its quality. You have not provided evidence that asbestos contaminated talc-containing cosmetic products are currently being marketed, since the data submitted is almost 40 years old.

Because safety questions about the possible presence of asbestos in talc are raised periodically, in 2009 FDA conducted an exploratory survey of currently marketed cosmetic-grade raw material talc and finished cosmetic products containing talc. This survey analyzed cosmetic-grade raw material talc from four suppliers out of a possible group of nine suppliers we had requested talc samples from, along with thirty-four talc-containing cosmetic products currently available in the Washington, D.C. metropolitan area for the presence of asbestos. In order to cover as broad a product range as possible, samples identified for testing included low, medium, and high priced products, along with some from "niche" markets. The cosmetic products identified as containing talc included eye shadow, blush, foundation, face powder, and body powder.

The survey found no asbestos fibers or structures in any of the samples of cosmetic-grade raw material talc or cosmetic products containing talc. While FDA found this data informative, the results were limited by the fact that only four suppliers submitted samples and by the number of products tested. They do not prove that all talc-containing cosmetic products currently marketed in the United States are free of asbestos contamination. As always, when potential public health concerns are raised, we will continue to monitor for new information and take appropriate actions to protect the public health. You may wish to see more on this survey on our website at http://www.fda.gov/Cosmetics/ProductandIngredientSafety/SelectedCosmeticIngredients/ucm293184.htm.

Toxicology Findings:

Your second major point is that talc is a carcinogen with or without the presence of asbestos-like fibers. The basis to this claim is that in 1993, the National Toxicology Program (NTP) published a study on the toxicity of non-asbestiform talc and found clear evidence of carcinogenic activity.

This NTP report concluded that cosmetic-grade talc caused tumors in animals, even though no asbestos-like fibers were found. The report made the following observations:

- There was some evidence of carcinogenic activity in non-asbestiform talc from inhalation studies in male rats based on an increased incidence of benign or malignant pheochromocytomas of the adrenal gland.
- There was clear evidence of carcinogenic activity of talc in female rats based on increased incidences of alveolar/bronchiolar adenomas and carcinomas of the lung and benign or malignant pheochromocytomas of the adrenal gland.
- There was no evidence of carcinogenic activity of talc in male or female mice exposed to 6 or 18 mg/cubic meter.

However, this study lacks convincing scientific support because of serious flaws in its design and conduct, including:

- The investigators used micronized talc instead of consumer-grade talc resulting in the experimental protocol not being reflective of human exposure conditions in terms of particle size.

- Investigators conceded that they had problems with the aerosol generation system; whereby, the target aerosol concentrations were either excessive or not maintained during 26 of the 113-122 weeks of the study.
- The study did not include positive and negative dust controls which would have permitted an "exact assessment" of the talc's carcinogenicity relative to the two control dusts.

In light of these shortcomings, a panel of experts at the 1994 ISRTP/FDA workshop declared that the 1993 NTP study has no relevance to human risk.

In addition, we reviewed relevant toxicity literature (consisting of 15 articles from 1980 to 2008), not cited in your Petitions, to determine if there was additional support at this point in time to for your suggested warning label. Scientific literature on studies of acute exposure effects, subchronic exposure effects, chronic exposure or carcinogenicity effects, developmental or reproductive toxicity, and genotoxicity effects were reviewed. As a result of the review of this relevant literature, FDA did not find enough additional support at this point in time for your suggested warning label.

Epidemiology and Etiology Findings:

Your third major point is that epidemiological studies confirm the causal relation between genital application of talc and ovarian cancer, and the protective effect of tubal ligation or hysterectomy, preventing the translocation of talc to the ovary.

After consideration of the scientific literature submitted in support of both Citizen Petitions, FDA found:

1 The exposure to talc is not well-characterized; it is not known if the talc referred to in the scientific studies was free of asbestos contamination; various consumer brands or lots of talc were not identified; and contamination of talc by asbestiform minerals or other structurally similar compounds was not ruled out.

2 Several of the studies acknowledge biases in the study design and no single study has considered all the factors that potentially contribute to ovarian cancer, including selection bias and/or uncontrolled confounding that result in spurious positive associations between talc use and ovarian cancer risk.

3 Results of case-controls studies do not demonstrate a consistent positive association across studies; some studies have found small positive associations between talc and ovarian cancer but the lower confidence limits are often close to 1.0 and dose-response evidence is lacking.

4 A cogent biological mechanism by which talc might lead to ovarian cancer is lacking; exposure to talc does not account for all cases of ovarian cancer; and

5 there was no scientific consensus on the proportion of ovarian cancer cases that may be caused by talc exposure.

6 The conclusion of the International Agency for Research on Cancer that epidemiological studies provide limited evidence for the carcinogenicity of perineal use of talc based body powder and the IARC classification of body-powder talc as group-2B, a possible carcinogen to human beings, is persuasive, but the results of the Nurses' Health Study, a large prospective cohort study, revealed no overall association with ever talc use and epithelial ovarian cancer.

Per the etiology review, approximately 10% of epithelial ovarian cancers are associated with inherited mutations. The remaining 90% of epithelial ovarian cancers are not related to these genetic mutations are non-hereditary. They have been historically classified based on histology as borderline/low malignant potential, serous, endometrioid, mucinous, and clear-cell.

Two theories have historically dominated on the cause of epithelial ovarian cancer and these are the "incessant ovulation hypothesis" and the "gonadotropin hypothesis." In addition to these endogenous factors, the role of exogenous factors via retrograde transport of noxious substances (e.g. carcinogens, particulates such as talc and asbestos, endometriosis and infectious agents) from the vagina and uterus into the Fallopian Tubes and peritoneal cavity have been studied extensively as a possible risk factor for ovarian cancer.

While there exists no direct proof of talc and ovarian carcinogenesis, the potential for particulates to migrate from the perineum and vagina to the peritoneal cavity is indisputable. It is, therefore, plausible that perineal talc (and other particulate) that reaches the endometrial cavity, Fallopian Tubes, ovaries and peritoneum may elicit a foreign body type reaction and inflammatory response that, in some exposed women, may progress to epithelial cancers. However, there has been no conclusive evidence to support causality.

The best evidence for an association or causal relationship between genital talc exposure and ovarian cancer comes from epidemiologic data which show a statistically significant but modest increased risk of epithelial ovarian cancer, especially with serous histology, among women with a history of genital dusting with talcum powder. While the growing body of evidence to support a possible association between genital talc exposure and serous ovarian cancer is difficult to dismiss, the evidence is insufficient for FDA to require as definitive a warning as you are seeking.

Request for hearing

In addition to your request for a warning label, you also requested a hearing, under 21 CFR 10.30(h)(2), so that you can present scientific evidence in support of your petitions.

Under this regulation, FDA may deny a citizen petition request for a hearing if the data and information submitted (even if accurate), are insufficient to justify the determination urged. In consideration of your request, we conducted an expanded literature search dating from the filing of the petition in 2008 through January 2014. The results of this search failed to identify any new compelling literature data or new scientific evidence.

Since we find that the data and information are insufficient to justify the determination you request and we did not identify any new compelling literature data or new scientific evidence, FDA is also denying your hearing request.

II. Conclusion

FDA appreciates the goals of the Cancer Prevention Coalition and FDA supports the goal of reducing the rate of ovarian cancer. Although FDA is denying the Cancer Prevention Coalition's petitions for the reasons discussed above, the Agency shares your commitment to the public health.

Sincerely,

Steven M. Musser, Ph.D.
Deputy Director for Scientific Operations
Center for Food Safety
and Applied Nutrition

Drafted: J. Gasper, OCAC, 2/28/14
Comments: L. Katz, OCAC, 3/3/14
Revised: J. Gasper, OCAC, 3/4/14
Cleared: N.Sadrieh, OCAC, 3/4/14
Cleared: LMKatz, OCAC, 3/5/14
Reviewed: FHogue, OCAC: 3/614
Cleared by:Musser:3/13/14
F/T:SRussell, OCAC 3/18/14

Author Manuscript Published OnlineFirst on April 14, 2015; DOI: 10.1158/1055-9965.EPI-15-0023
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

African-Americans and Hispanics remain at lower risk of ovarian cancer than non-Hispanic Whites after considering non-genetic risk factors and oophorectomy rates

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Running Title: Ethnicity and Ovarian Cancer Risk

Key words: ovarian cancer, population attributable risk percentages, ethnicity

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Disclosure of Potential Conflicts of Interest: None

Grant Support: This work was supported by grants from the National Cancer Institute (CA58598, CA17054) (Pike, Wu, Pearce), the California Cancer Research Program (2II0200) (Wu, Pike, Tseng), as well as Cancer Center Core Grants awarded to the University of Southern California (USC) and Memorial Sloan Kettering (MSK) (P30 CA014089, P30 CA008748) from the National Cancer Institute. The collection of incident ovarian cancer cases for this study by the USC Cancer Surveillance Program (CSP) is partly supported under subcontract by the California Department of Health. The CSP is also part of the National Cancer Institute's Division of Cancer Prevention and Control's Surveillance, Epidemiology, and End Results (SEER) Program, under contract number N01CN25403.

Author Manuscript Published OnlineFirst on April 14, 2015; DOI: 10.1158/1055-9965.EPI-15-0023
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Abstract (250 words)

Background Risk factors for invasive epithelial ovarian cancer (IEOC) among Hispanics and African Americans are under-studied despite notable differences in incidence relative to non-Hispanic Whites.

Methods We used multivariate logistic regression to examine parity, oral contraceptive use, tubal ligation, endometriosis, family history of ovarian cancer, and talc use and risk of IEOC among Hispanics (308 cases, 380 controls), African Americans (128 cases, 143 controls) and non-Hispanic Whites (1265 cases, 1868 controls) using four case-control studies we conducted in Los Angeles County. We expressed each of these factors in the form of increasing risk and calculated population attributable risk percentage (PAR%) estimates for the six risk factors separately and jointly in the three groups.

Results The risk associations with these six well-accepted factors were comparable in the three groups. The significant racial/ethnic differences in the prevalence of these factors and differences in their oophorectomy rates explained 31% of the lower incidence in African Americans compared to non-Hispanic Whites, but only 13% of the lower incidence in Hispanics. The PAR%s ranged from 27.5% to 31.0% for no tubal ligation, 15.9% to 22.2% for not using oral contraceptives, and 12.2% to 15.1% for using talc in the three groups.

Conclusions All six risk factors are comparably important in the three groups. Differences in the prevalence of these factors and their oophorectomy rates explained approximately one-third of the difference in incidence between African Americans and non-Hispanic Whites.

Impact Devising strategies to lessen the burden of IEOC will be applicable to all three racial/ethnic groups.

Author Manuscript Published OnlineFirst on April 14, 2015; DOI: 10.1158/1055-9965.EPI-15-0023
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Introduction

In the US in the period 2000-2009, the annual age-adjusted incidence rate of invasive epithelial ovarian cancer (IEOC) was highest in non-Hispanic Whites (14.3 per 100,000), intermediate in Hispanics (12.1 per 100,000; 15% lower than the rate in non-Hispanic Whites) and lowest in African Americans (10.2 per 100,000; 29% lower than the rate in non-Hispanic Whites) (1). Epidemiologic studies of ovarian cancer risk have focused primarily on non-Hispanic White women; reasons for the racial/ethnic differences in incidence are not well understood.

A number of risk factors –first degree family history of ovarian cancer, endometriosis, and use of talc – and protective factors – parity, use of oral contraceptives, and tubal ligation – have been unequivocally associated with ovarian cancer in non-Hispanic Whites. There is virtually no information on ovarian cancer risk factors in Hispanics. A small number of Hispanic cases (n=42) were included in an ovarian cancer case-control study conducted in the Central Valley of California, but only results on talc use were reported separately in Hispanics (35.7% in cases vs 26.9% in controls) (2). A hospital-based case-control study in Mexico compared risk factors between 84 ovarian cancer cases and control women selected from an outpatient clinic (3): parity and use of oral contraceptives were significantly inversely associated with risk but information on other factors has not been presented.

Risk factors for ovarian cancer among African Americans have been examined in three reports (4-6). The Collaborative Analysis of US Case-Control Studies of Ovarian Cancer included seven studies with a total of 110 ovarian cancers (72 invasive, 35 borderline, 3 unknown) in African-American women (4). Ness and colleagues reported on risk of ovarian cancer among 84 African-American women with invasive or borderline cancers (numbers of each not specified) from their Delaware Valley case-control study (5). More recently, Moorman and colleagues reported results from 111 African Americans with invasive ovarian cancer from their North Carolina ovarian cancer case-control study (6). Reduced risk from increased parity and oral contraceptive use were found in all three studies. Tubal ligation was found

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to be significantly inversely associated with risk in both of the studies that reported on this factor (5, 6). The results regarding family history are unclear. John and colleagues did not report on family history (4). Ness and colleagues found that a family history of ovarian cancer was inversely associated with risk in African Americans but this was based on sparse numbers (1.2% of cases vs 2.0% of controls), a finding contrary to the strong increased risk found in non-Hispanic Whites (4.6% of cases vs 1.9% of controls)(5). Family history of ovarian cancer was not reported in the North Carolina study but family history of breast or ovarian cancer was a significant risk factor for African Americans (6).

The literature on causes of IEOC in Hispanics and African Americans is therefore very limited and it remains unclear to what extent the differences in the prevalence of ovarian cancer risk factors explain the differences in incidence between these three racial/ethnic groups. During the period 1992-2008, we conducted four IEOC case-control studies in Los Angeles County designed to elucidate risk factors for the disease and to evaluate differences in risk across non-Hispanic Whites, Hispanics, and African Americans.

Materials and Methods

The results presented here are based on pooling the questionnaire data from these four studies which used identical data collection methods as regards the factors discussed here; comprehensive details of these methods have been published (7-9). These studies were approved by the University of Southern California Institutional Review Board, and written informed consent was obtained from each patient and control before her interview.

Case Ascertainment

For all studies, newly diagnosed histologically confirmed IEOC cases were identified from the USC Cancer Surveillance Program which is the Los Angeles County SEER Program. Eligible patients were female residents of Los Angeles County of self-reported non-Hispanic White, Hispanic, or African-American race/ethnicity. Cases were eligible for inclusion in the study if they were between 18 and 74

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years of age at diagnosis (up to age 79 for cases diagnosed between 2003 and 2008). A total of 3,370 patients met the study criteria (2,580 non-Hispanic Whites, 506 Hispanics, 284 African Americans). Overall, 15.7% of patients (17.2% non-Hispanic Whites, 8.5% Hispanics, 15.5% African Americans) declined to be interviewed, 16.9% had died or were too ill to be interviewed (17.8% non-Hispanic Whites, 12.1% Hispanics, 17.6% African Americans), and 11.4% could not be located or had moved out of Los Angeles County (10.2% non-Hispanic Whites, 14.0% Hispanics, 17.6% African Americans). We were thus able to carry out in-person interviews with 1,886 patients (1,415 non-Hispanic Whites, 331 Hispanics, and 140 African Americans), representing 63.2% participation rate of the patients approached (61.1% non-Hispanic Whites, 76.1% Hispanics, and 59.8% African Americans). The response rate was higher for patients diagnosed with localized cancer (69%) compared to those with more advanced stage at diagnosis (61%). Response rates were highest for those diagnosed under age 60 (70%), intermediate for those ages 60-69 (59%), and lowest for those ages 70+ (47%) at diagnosis. In this analysis, we excluded 185 patients who had a previous cancer (excluding non-melanoma skin cancer) or had prior bilateral oophorectomy and the final analysis was based on 1,701 patients (1,265 non-Hispanic Whites, 308 Hispanics, and 128 African Americans).

Control Ascertainment

Controls were residents of Los Angeles County with at least one intact ovary identified using a well-tested neighborhood control selection algorithm (8-10). Neighborhood controls were individually matched to cases on race/ethnicity and year of birth (+/- 5 years); they represented essentially all the controls interviewed. In one study, selection of controls for cases >65 years of age was augmented, if necessary, by using lists of female residents of Los Angeles County provided by the Health Care Financing Administration, matched to the case on zip code, race/ethnicity, and year of birth closest to the case's year of birth (8). Overall, 70% of the non-Hispanic White, Hispanic, and African-American controls interviewed were the first identified control.

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Data Collection

In-person interviews were conducted using standardized questionnaires which included the use of a life calendar. The core questions on the risk factors presented here were identical in the four studies. The questionnaire covered events up to 12 months before a case's diagnosis date and a similar reference date for the controls.

The demographic, lifestyle, and medical history variables considered in this analysis include race/ethnicity (African American, Hispanic, non-Hispanic White), age at diagnosis, parity, oral contraceptive use, tubal ligation, self-reported physician-diagnosed endometriosis, first degree family history of ovarian cancer, and genital talc use.

Statistical Analysis

We employed standard statistical methods including multivariate logistic regression using the statistical package programs STATA 12 (StataCorp, College Station, TX) and SAS 9.2 (SAS Institute Inc., Cary, NC). Although the studies were designed as matched case-control studies, at the termination of the particular studies, some cases had not been matched to a control and there were some controls whose cases had to be excluded after they completed the interview, because they were ineligible for the current analysis (*e.g.*, not IEOC or did not live in Los Angeles County at the time of diagnosis). In this report we have used all interviewed cases and controls by adopting a stratified multivariate logistic regression analysis approach with joint stratification for the three race/ethnicity groups, age group (<30, five year age groups to age 79), interviewer, and study. Analysis focused on the following factors: nulliparity (yes/no), oral contraceptive use (yes/no; no included never and <1 year of use), tubal ligation (yes/no), history of endometriosis (yes/no), family history of ovarian cancer (mother or sister; yes/no), and history of genital talc use (yes/no; no included never and <1 year of use). The logistic regression analysis also adjusted for menopausal status (premenopausal, natural menopause age ≤49, natural menopause age 50-54, natural menopause ≥55, surgical menopause (simple hysterectomy only) age ≤49,

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surgical menopause ≥50, other), age at menarche (≤11, 12, 13, ≥14), hormone therapy use (none, former or current estrogen + progestin, former or current estrogen alone), body mass index (BMI; kg/m^2) (≤22, >22-24, >24-28, >28), family income (≤40,000, >40,000 to ≤64,000, >64,000 to ≤100,000, >100,000, don't know) and education (high school or less, some college, college or higher). Odds ratios (ORs) - and corresponding 95% confidence intervals (CIs) - were calculated as estimates of the relative risks (RRs). All statistical significance values (P values) quoted are two-sided.

Population attributable risk percentages (PAR%s), defined as the percentages of disease in the population that are attributable to a given risk factor (or set of risk factors), were calculated using the method of Bruzzi *et al.* (11). These authors showed that PAR%s could be calculated from a case-control study using the estimated RRs applied to the cases only. This approach is of particular value to our analysis as it only requires the cases to be a representative sample from the population at risk. This method uses the individual data on each case to calculate the expected fraction of the cases that would not have occurred if the risk factors being considered were at their baseline values, and this fraction was then used to calculate the PAR%. For a single risk factor the confidence limit for the PAR% was obtained by repeating the calculation using the lower (and upper) confidence bound of the OR for the particular factor in this calculation. For multiple risk factors, the confidence bounds for the PAR% were obtained by simulation: drawing repeated random samples from the mean and covariance matrix of the log ORs from the logistic regression fit and calculating a PAR% from that sample - the 95% confidence bounds were taken as the 2.5% and 97.5% values from the repeated samples. In our simulation analyses we used 5,000 repeats.

Published incidence rates for IEOC make no adjustment for the number of women who have had their ovaries (and fallopian tubes) removed. Writing h for the proportion of women who have had a hysterectomy and t for the proportion of hysterectomies that include removal of the ovaries (oophorectomy), an incidence rate r is approximately adjusted (not accounting for age at oophorectomy)

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for the oophorectomy rate as follows:

Formula (A) $r_{\text{adj-ooph}} = r/(1 - h \times t)$

If a population incidence rate (or an oophorectomy adjusted incidence rate) r is associated with a PAR% p for a single risk factor (or a group of risk factors) then the expected incidence rate if the population was at the baseline risk of the risk factor is:

Formula (B) $r_{\text{adj-PAR\%}} = r \times (1 - \text{p}/100)$

Results

This analysis was based on 1701 women diagnosed with IEOC (1265 non-Hispanic Whites, 308 Hispanics, and 128 African Americans) and 2391 control women (1868 non-Hispanic Whites, 380 Hispanics, and 143 African Americans). The distribution of IEOC by histology, stage at diagnosis and differentiation did not differ significantly between the three groups (Table 1). The majority of IEOC in the three racial/ethnic groups was of serous cell type, distant stage at diagnosis, and poorly differentiated.

The prevalence of the risk factors including the average number of births, duration of oral contraceptive use, and duration of talc use in the three groups of controls and cases are shown in Table 2. All six factors are presented in the manner of being associated with increasing risk; *i.e.*, the factors that are inversely associated with risk are presented in the form of their absence being a risk factor, *e.g.*, the decreased risk in parous women is presented as a risk in nulliparous women. This was done to allow the presentation of PAR%s in a standard fashion.

With the exception of family history of ovarian cancer, the prevalence of the other risk factors differed significantly between the three racial/ethnic groups of control women (Table 2, top). The prevalence of no tubal ligation was 69.2% in African-American, 73.7% in Hispanic, and 85.9% in non-Hispanic White control women (P_{2df}<0.0001). Nulliparity and history of endometriosis was highest in non-Hispanic Whites, intermediate in African Americans, and lowest in Hispanics (23.7%, 16.8% and 13.7% for nulliparity, P_{2df}<0.001; 7.5%, 5.6% and 3.4% for endometriosis, P_{2df}=0.008). No oral

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contraceptive use (no/<1 year) was highest in Hispanics (54.7%), followed by African Americans (47.6%), and lowest in non-Hispanic Whites (41.5%) (P_{2df} <0.001). Talc use was more common in African-American women (44.1%) than in non-Hispanic Whites (30.4%) or Hispanics (28.9%) (P_{2df} =0.001). Similar patterns of differences in these risk factors between the three racial/ethnic groups of IEOC patients were found (Table 2, bottom).

As expected, each of the six risk factors had statistically significant independent effects on risk in non-Hispanic Whites. Risk patterns in Hispanics paralleled those in non-Hispanic Whites (Table 3), although the elevated risks with endometriosis and family history of ovarian cancer did not achieve statistical significance. In African Americans, family history of ovarian cancer was associated with a more than 7-fold increased risk, but the confidence interval was wide (OR=7.84, 95% CI=1.66-37.0). The associations with parity, oral contraceptive use, tubal ligation, endometriosis, and talc use in African Americans are all in agreement with the risks found in non-Hispanic Whites, although none were statistically significant. The adjusted ORs for the three racial/ethnic groups combined are also shown in Table 3.

The first three columns of Table 4 show that these six factors together accounted for 57.9% of IEOCs in non-Hispanic Whites compared with 56.1% in Hispanics and 53.8% in African Americans based on the race/ethnicity-adjusted OR estimates shown in Table 3 (last column). The PAR% due to 'no tubal ligation' was large in all three racial/ethnic groups, ranging from 27.5% to 31.0%, followed by 'no oral contraceptive use' (ranging from 15.9% to 22.2%), and talc use (ranging from 12.2% to 15.1%). The PAR% for nulliparity was 8.9% in non-Hispanic Whites, but lower in Hispanics (5.7%) and African Americans (5.5%). The PAR%s for endometriosis (ranging from 2.0% to 4.0%) and family history of ovarian cancer (ranging from 2.7% to 3.9%) were more modest. The large 'no tubal ligation' PAR% is due to relatively high prevalence in the IEOC patients (Table 2, bottom); it was 90.6% in non-Hispanic Whites, 83.8% in Hispanics, and 80.5% in African Americans, so that a shift to the low-risk category, *i.e.*, having a tubal ligation, will have a substantial impact. In contrast, the PAR% due to nulliparity is lower

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because being parous is already highly prevalent; 72.2% in non-Hispanic Whites, 83.6% in African Americans, and 82.1% in Hispanics, so that a shift to the low-risk category will have a lesser impact on the overall disease burden.

The mean number of births among parous IEOC cases was 2.5 in non-Hispanic Whites, 2.8 in African Americans and 3.1 in Hispanics (Table 2, bottom). We repeated the PAR% calculations after categorizing births as 0, 1, 2, 3 and 4+ using the 4+ category as baseline: the associated PAR% values increased as expected but the relationships of the PAR%s by racial/ethnic group were essentially unaltered. Similarly, we categorized oral contraceptive use in finer categories of <1 year, 1-4 years, 5-9 years and 10+ years with little effect on the relationships of the PAR%s by racial/ethnic group (data not shown).

Discussion

With the high mortality and the lack of effective early screening for ovarian cancer, better understanding of preventive risk factors is a priority. The primary motivation for this analysis was to determine whether the six confirmed non-genetic risk factors for IEOC (parity, use of oral contraceptives, tubal ligation, endometriosis, first degree family history of ovarian cancer, use of genital talc) in non-Hispanic Whites are also risk factors in Hispanics and African Americans. The risk patterns associated with these six factors were comparable in the three racial/ethnic groups (Table 3), and the PAR%s for the factors jointly (Table 4) were also very similar.

An additional objective was to determine whether these six risk factors jointly could explain the 29% and 15% lower incidence of ovarian cancer in African Americans and Hispanics, respectively, compared to non-Hispanic Whites. The incidence of ovarian cancer as reported by SEER, and other cancer registries, is calculated by considering all women in the denominator (population at risk) without removing those who have had a bilateral oophorectomy and are not at risk. Thus, estimates of

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Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

racial/ethnic differences in IEOC based on SEER data can be 'improved' by accounting for the racial/ethnic differences in the prevalence of bilateral oophorectomy.

While Lowder *et al.* (12) in their analysis of oophorectomy rates in women undergoing a hysterectomy in the National Hospital Discharge Survey covering the period 1979-2004, found that the proportion was approximately 40% and did not differ by racial/ethnic group; Jamison *et al.* (13) in their analysis of hysterectomy prevalence in women over age 50 in the Behavioral Risk Factor Surveillance System covering the years 1992-2008 found that the rate of hysterectomy was clearly higher in African-American women (47%) than in non-Hispanic Whites (41%) and lower still in Hispanic women (36%). Using figures from these two studies in *Formula (A)* (see Statistical Analysis section of the Methods section) to adjust incidence rates for the proportion of women with a history of oophorectomy, we estimate that the observed 29% lower incidence rate in African Americans compared to non-Hispanic Whites based on SEER data would be adjusted to 27% [= 1 - 0.71 × (1 - 0.41 × 0.4)/(1 - 0.47 × 0.4)]. The PAR% of non-Hispanic Whites was slightly higher at 57.8% than the PAR% in African Americans at 53.8% (Table 4); taking this into account, by use of *Formula (B)* (see Statistical Analysis section of the Methods section), reduced the difference in incidence between the two groups further from the adjusted 27% to 20%. Overall, taking into account the correction in the population at risk (denominator) and the PAR%, the difference in the African-American to non-Hispanic White incidence rates was reduced by 31% (1-20%/29%). Given that hysterectomy rates are lower in Hispanics compared to non-Hispanic Whites, Hispanics would be at even lower relative risk than what is observed in SEER; the 15% lower incidence rate in Hispanics compared to non-Hispanic Whites would increase to 17% when using the correct at-risk denominator. The PAR% difference will change the difference slightly less in Hispanics compared to non-Hispanic Whites from 17% to 13%. When taking into consideration the correct population at risk and the PAR%, the difference in incidence rates between Hispanics and non-Hispanic Whites is reduced by 13% (1-13%/15%). Thus this type of analysis suggests that further investigations

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are needed to identify other risk factors that may explain the remaining differences in IEOC rates between these three racial/ethnic groups.

Strengths of this study include the ability to evaluate the relative comparability in the effect of several well-established risk factors in non-Hispanics Whites, Hispanics and African Americans. Our results on Hispanics fill a knowledge gap, as this is the first study to examine etiologic risk factors for ovarian cancer in this growing minority population in the US. Identical questionnaires and protocols were used in these four studies. Although information on these six factors was based on self-report, there is no evidence of systematic misclassification bias as the direction of racial/ethnic differences in the prevalence of tubal ligation, use of oral contraceptives, and endometriosis are consistent with other studies (6, 14-16). However, these results must be considered with caution as we were limited in that the sample sizes of Hispanics and African Americans were modest and we investigated only the six factors that are confirmed, noncontroversial, showing strong associations with all invasive ovarian cancers in non-Hispanic Whites. The modest sample sizes precluded us from conducting analyses separately by histologic type. The response rate for the three racial/ethnic groups was also modest, but not unlike the response rate for other case-control studies on ovarian cancer.

The comparable risk factor associations in IEOC in African Americans, Hispanics, and non-Hispanic Whites contrast sharply with the more disparate risk factor patterns in breast cancer by race/ethnicity. A number of factors that are known to affect breast cancer risk in non-Hispanic Whites do not appear to influence risk in African Americans and these differences cannot be explained by different prevalence of estrogen receptor/progesterone receptor positive breast tumors between the two groups (17-21). Breast cancer risk factors also appeared to differ profoundly between Hispanics and non-Hispanic Whites in one of the few studies with comparable data on both race/ethnic groups (15). Given the more comparable risk factor patterns in IEOC for non-Hispanic Whites, Hispanics, and African Americans, devising strategies to lessen the burden of IEOC will be applicable to all groups.

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Summary

Results from these population-based case-control studies suggest that the six well-established risk factors for IEOC accounted for about 60% of ovarian cancer risk in non-Hispanic Whites, Hispanics and African Americans. There were differences in the prevalence of these factors in the different racial/ethnic groups, and the 27% lower incidence of ovarian cancer in African Americans compared to non-Hispanic Whites was reduced to 20% when these differences were adjusted for, but adjustment for these differences in prevalence accounted for only a very small amount of the lower incidence rate in Hispanics.

Acknowledgment

We would like to express our sincere thanks to our interviewers and the other members of our data collection teams. Most importantly we are most grateful to all the study participants for volunteering for these studies and providing us with their contributions and support. The ideas and opinions expressed herein are those of the authors, and endorsement by the State of California, the California Department of Health Services, the National Cancer Institute, or the Centers for Disease Control and Prevention or their contractors and subcontractors is not intended nor should be inferred.

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Author Manuscript Published OnlineFirst on April 14, 2015; DOI: 10.1158/1055-9965.EPI-15-0023
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Table 1. Tumor characteristics of invasive ovarian cancer in non-Hispanic Whites, Hispanics, and African Americans: Los Angeles County Ovarian Cancer Study

	non-Hispanic Whites N=1265	Hispanics N=308	African Americans N=128
Age			
<30	12 (0.9%)	5 (1.6%)	1 (0.8%)
30-34	14 (1.1%)	11 (3.6%)	2 (1.6%)
35-39	33 (2.6%)	10 (3.2%)	3 (2.3%)
40-44	58 (4.6%)	31 (10.1%)	13 (10.2%)
45-49	144 (11.4%)	36 (11.7%)	17 (13.3%)
50-54	194 (15.3%)	60 (19.5%)	25 (19.5%)
55-59	186 (14.7%)	46 (14.9%)	18 (14.1%)
60-64	193 (15.3%)	43 (14.0%)	24 (18.8%)
65-69	179 (14.2%)	29 (9.4%)	15 (11.7%)
70-74	160 (12.6%)	26 (7.5%)	8 (6.3%)
75-79	92 (7.3%)	14 (4.5%)	2 (1.6%)
Histology			
Serous	721 (57.0%)	179 (58.1%)	71 (55.5%)
Mucinous	85 (6.7%)	26 (8.4%)	12 (9.4%)
Endometrioid	153 (12.1%)	34 (11.0%)	14 (10.9%)
Clear-cell	75 (5.9%)	14 (4.5%)	4 (3.1%)
Epithelial	40 (3.2%)	13 (4.2%)	2 (1.6.%)
Undifferentiated/poorly	53 (4.2%)	12 (3.9%)	10 (7.8%)
Other	131 (10.4%)	28 (9.1%)	14 (10.9%)
Not known	7 (0.6%)	2 (0.6%)	1 (0.8%)
P_{3df} [ab]		0.54	0.40
Stage			
Localized	216 (17.1%)	58 (18.8%)	30 (23.4%)
Regional	170 (13.4%)	49 (15.9%)	12 (9.4%)
Distant	853 (67.4%)	197 (64.0%)	83 (64.8%)
Not known	26 (2.1%)	4 (1.3%)	3 (2.3%)
P_{2df} [ac]		0.38	0.12
Differentiation			
Well	119 (9.4%)	29 (9.4%)	9 (7.0%)
Mod well	235 (18.6%)	53 (17.2%)	28 (21.9%)
Poorly	502 (39.7%)	119 (38.6%)	46 (35.9%)
Undifferentiated	170 (13.4%)	33 (10.7%)	16 (12.5%)
Not known	239 (18.9%)	74 (24.0%)	29 (22.7%)
P_{3df} [ab]		0.81	0.63

[a] P value comparing non-Hispanic Whites to each of the other two groups separately.
[b] P value based on cases of serous, mucinous, endometrioid and clear-cell histology only.
[c] P value excluding cases with not known histology or stage of cancer at diagnosis.

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Table 2. Prevalence of risk factors in non-Hispanic White, Hispanic and African-American control women (top) and ovarian cancer cases (bottom)

Factors	Non-Hispanic Whites	Hispanics	African Americans	P1[b]	P2[c]	P3[d]
			Controls[a]			
% Nulliparous	23.7%	13.7%	16.8%	<0.001	0.076	0.45
Mean # births among parous (SD)	2.5 (1.3)	3.0 (1.7)	2.7 (1.5)	<0.001	0.03	0.15
% Oral contraceptive use (no/< 1 year)	41.5%	54.7%	47.6%	<0.001	0.19	0.17
Mean # months of OC use among users (SD)	95.9 (74.9)	81.0 (67.0)	93.1 (74.2)	0.014	0.75	0.21
% No tubal ligation	85.9%	73.7%	69.2%	<0.001	<0.001	0.36
% Endometriosis	7.5%	3.4%	5.6%	0.006	0.50	0.38
% Family history of ovarian cancer	2.5%	3.4%	2.8%	0.37	0.98	0.93
% Talc use ≥1 year	30.4%	28.9%	44.1%	0.61	0.0001	0.002
Mean # years of talc use among users (SD)	23.9 (17.4)	21.3 (16.7)	22.9 (17.0)	0.15	0.67	0.55
			Cases[d]			
% Nulliparous	27.8%	17.9%	16.4%	<0.001	0.007	0.82
Mean # births among parous (SD)	2.5 (1.2)	3.1 (1.7)	2.8 (1.6)	<0.001	0.003	0.24
% Oral contraceptive use (no/< 1 year)	57.4%	69.8%	50.0%	<0.001	0.13	<0.001
Mean # months of OC use among users (SD)	73.4 (61.1)	59.8 (53.1)	75.7 (66.7)	0.044	0.75	0.10
% No tubal ligation	90.6%	83.8%	80.5%	<0.001	<0.001	0.49
% Endometriosis	11.1%	5.5%	9.4%	0.005	0.66	0.21
% Family history of ovarian cancer	5.1%	4.9%	7.0%	0.96	0.48	0.50
% Talc use ≥1 year	41.2%	38.6%	47.7%	0.45	0.19	0.10
Mean # years of talc use among users (SD)	27.5 (18.4)	21.6 (16.9)	26.6 (18.2)	0.001	0.71	0.069

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Abbreviation: SD, standard deviation

[a] Controls included: 1,868 Non-Hispanic Whites, 380 Hispanics, and 143 African Americans

[b] P_{1df} for differences between non-Hispanic Whites and Hispanic controls (top)/ P_{1df} for differences between non-Hispanic Whites and Hispanic cases (bottom)

[c] P_{1df} for differences between non-Hispanic whites and African American controls (top)/ P_{1df} for differences between non-Hispanic whites and African American cases (bottom)

[d] P_{1df} for differences between Hispanic and African American controls (top)/ P_{1df} for differences between Hispanic and African American cases (bottom)

[e] Cases included: 1,265 Non-Hispanic Whites, 308 Hispanics, and 128 African Americans

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Table 3. Mutually adjusted odds ratios[a] for invasive ovarian cancer in Los Angeles County non-Hispanic Whites, Hispanics, and African Americans

	non-Hispanic Whites (1,265/1,868)		Hispanics (308/380)		African Americans (128/143)		All (1701/2391)	
	ca/co	OR (95% CI)	ca/co	OR (95% CI)	ca/co	OR (95% CI)	ca/co	OR (95% CI)
Livebirths								
Yes	913/1426	1.00	253/328	1.00	107/119	1.00	1273/1873	1.00
No	352/442	1.43 (1.19-1.73)	55/52	2.22 (1.28-3.84)	21/24	1.42 (0.54-3.75)	428/518	1.47 (1.24-1.75)
Per birth		0.70 (0.58-0.84)		0.45 (0.26-0.78)		0.70 (0.27-1.86)		0.68 (0.57-0.81)
Oral Contraceptive (OC)								
Yes	539/1092	1.00	93/172	1.00	64/75	1.00	696/1339	1.00
None/<1 yr	726/776	1.55 (1.31-1.84)	215/208	1.29 (0.87-1.92)	64/68	1.30 (0.64-2.63)	1005/1052	1.47 (1.26-1.70)
Per 5 yrs OC		0.64 (0.54-0.76)		0.77 (0.52-1.15)		0.77 (0.38-1.55)		0.68 (0.59-0.79)
Tubal ligation								
Yes	119/263	1.00	50/100	1.00	25/44	1.00	194/407	1.00
No	1146/1605	1.41 (1.10-1.81)	258/280	1.71 (1.07-2.74)	103/99	1.65 (0.73-3.74)	1507/1984	1.52 (1.23-1.87)
Endometriosis								
No	1125/1728	1.00	291/367	1.00	116/135	1.00	1532/2230	1.00
Yes	140/140	1.51 (1.15-1.98)	17/13	2.21 (0.89-5.48)	12/8	1.74 (0.45-6.74)	169/161	1.56 (1.21-2.00)
First degree family history of ovarian cancer								
No	1200/1822	1.00	293/367	1.00	119/139	1.00	1612/2328	1.00
Yes	65/46	2.12 (1.40-3.21)	15/13	2.38 (0.94-6.01)	9/4	7.84 (1.66-37.0)	89/63	2.26 (1.58-3.25)
Genital talc use								
None/<1 yr	744/1300	1.00	189/270	1.00	67/80	1.00	1000/1650	1.00
Yes	521/568	1.41 (1.21-1.67)	119/110	1.77 (1.20-2.62)	61/63	1.56 (0.80-3.04)	701/741	1.46 (1.27-1.69)
Per 5 yrs talc		1.14 (1.08-1.21)		1.18 (1.02-1.36)		1.15 (0.90-1.47)		1.14 (1.09-1.20)

[a] Multivariate logistic regression analyses were jointly stratified for race/ethnicity, age group (<30, five year age groups to age 79), interviewer and study, and adjusted for menopausal status, age at menarche, hormone therapy use, body mass index, income, education, and each of the six factors shown

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Table 4. Ovarian cancer population attributable risk percentages (PAR%s) in Los Angeles County non-Hispanic Whites, Hispanics, and African Americans[a]

	non-Hispanic Whites	Hispanics	African Americans
	Using race-adjusted ORs[a]		
	PAR%[b]	PAR%[b]	PAR%[b]
No livebirth	8.9% 5.3%-11.9%	5.7% 3.4%-7.6%	5.3% 3.1% - 7.0%
No/<1 yr oral contraceptives	18.3% 12.0%-23.7%	22.2% 14.5%-28.8%	15.9% 10.4% - 20.7%
No tubal ligation	31.0% 17.2%-42.3%	28.7% 15.9%-39.1%	27.5% 15.2% - 37.5%
Yes endometriosis	4.0% 2.0%-5.5%	2.0% 1.0%-2.8%	3.4% 1.7% - 4.7%
Yes family history ovarian cancer	2.9% 1.9%-3.6%	2.7% 1.8%-3.4%	3.9% 2.6% - 4.9%
Yes/≥1 yr talc use	13.0% 8.7%-16.8%	12.2% 8.1%-15.8%	15.1% 10.0% - 19.5%
3 factors (no tubal ligation, no/<1 yr oral contraceptives, yes/≥1 yr talc use)	50.8% 39.7%-59.5%	51.2% 40.8%-59.3%	47.9% 37.8%-55.8%
All 6 factors	57.9% 48.7%-65.3%	56.1% 46.8%-63.3%	53.8% 45.0% - 60.7%

[a] Using the all race/ethnicity adjusted ORs from Table 3.

[b] The PARs were mutually adjusted for the variables shown in this table as well as for race/ethnicity, age group (<30, five year age groups to age 79), interviewer and study, and adjusted for menopausal status, age at menarche, hormone therapy use, body mass index, income, and education.

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Cancer Epidemiology, Biomarkers & Prevention

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African Americans and Hispanics remain at lower risk of ovarian cancer than non-Hispanic Whites after considering non-genetic risk factors and oophorectomy rates

Anna H. Wu, Celeste Leigh Pearce, Chiu-Chen Tseng, et al.

Cancer Epidemiol Biomarkers Prev Published OnlineFirst April 14, 2015.

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Subject: URGENT: Talc Update

Global Baby Team-

Many of you have inquired about the recent U.S. talc litigation verdict **and I want to reassure everyone that we stand firmly behind the safety of the cosmetic talc we use in our products globally and will continue to defend against the allegations in the lawsuits that will continue in the U.S. throughout 2016 and beyond.** Our reactive media statement, attached below, has been shared with Regional Communication Leaders in APAC, LATAM, EMEA and N. America and the Communications team is monitoring across all mainstream and social media channels and responding as needed. In addition, the "Facts About Talc Safety" article, attached below, will be published in global ***Touchpoint*** on Wednesday, February 24. As the U.S. litigation continues in the weeks ahead, the team will continue to monitor and adapt our strategy to ensure that we are communicating with key global stakeholders about the body of science that supports the safety of talc.

It is also important for you to know that as part of our broader and ongoing global effort to educate external stakeholders, a cross-functional global team began working last year to assess our talc communications strategy for 2016 and beyond. Among the actions undertaken in 2015 were the implementation of a paid search strategy focused on talc safety and the addition of more consumer-friendly content to the talc section of the Safety website which is available in 13 languages.

Moving forward, it is critical that our core, cross-functional team manage ALL communications related to the U.S. litigation to ensure a consistent approach. If you receive media inquiries about the litigation, please contact your regional communications lead or the global media leader, Carol Goodrich at cgood2@its.jnj.com or (973) 615-4057. If you have other questions about the litigation, you should contact your law department representative. Thank you for your ongoing partnership.

Please reach out to me our Carol directly if any further questions here. We can also discuss, as a team, during our Innovation Sponsor Review on Thursday.

Debra

Reactive Global Media Statement:

"The talc used in all our global products is carefully selected and meets the highest quality, purity and compliance standards. The recent U.S. verdict goes against decades of sound science proving the safety of talc as a cosmetic ingredient in multiple products, and while we sympathize with the family of the plaintiff, we strongly disagree with the outcome. Ovarian cancer is a complex disease with no known cause and the U.S. Food and Drug Administration, National Cancer Institute and Cosmetic Ingredient Review Committee have all concluded that there is insufficient evidence linking talc to ovarian cancer."

Touchpoint article:

The Facts About Talc Safety

Baby Powder made from cosmetic talc is one of JOHNSON's oldest products and a longtime part of baby care rituals. JOHNSON'S Baby Powder continues to be popular with adults as well, and in many parts of the world, it remains an essential part of makeup and skin care routines. With over 100 years of use, few ingredients have the same demonstrated performance, mildness and safety profile as cosmetic talc.

Despite this legacy of safety, recent articles and online stories have misrepresented the facts and made inaccurate allegations about cosmetic talc and increased health risks, including cancer. In fact, some employees have told us they are getting questions from friends and family, many based on social media stories and attorney advertising with alarming headlines that are circulating online.

We want our employees to be well-informed about talc and its safety profile. In these days of easy access to conflicting messages via internet sites and social media discussions, it's important to know the facts.

FACT: JOHNSON's talc products do not contain asbestos. A frequent misperception is that JOHNSON'S Baby Powder contains talc made with asbestos, a substance classified as cancer-causing. We use only U.S. Pharmacopeia (USP) grade talc to ensure it meets the highest quality, purity and compliance standards. The talc used in all our global production is carefully selected and processed to be asbestos-free, which is confirmed by regular testing conducted since the 1970s.

FACT: The safety of talc is based on a long history of safe use and more than 30 years of research by independent researchers, scientific review boards and global authorities. Various agencies and governmental bodies have examined whether talc is a carcinogen and none have concluded that it is. No governing regulatory body has ever required a change in labeling to reflect any carcinogenic risk.

FACT: Among the agencies that have examined talc as a potential carcinogen are the U.S. Department of Health and Human Services and the U.S Food and Drug Administration (FDA). FDA reviewed the safety data on talc again in 2014 and stated they found "…no compelling new data or scientific evidence… " on the safety of talc.[1]

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FACT: The U.S. Center for Disease Control (CDC), which identifies potential risk factors for many diseases, has not identified talc as a risk factor for ovarian cancer.

FACT: Since the early 1990s, many research papers and epidemiology studies have evaluated talc and perineal use and these studies have found talc to be safe. In fact, the Nurses' Health Study (2010)[4] and the Women's Health Initiative Observational Cohort (2014)[5], the only two large-scale prospective studies looking at talc and ovarian cancer, found no causal relationship between talc and ovarian cancer.

FACT: Our talc products have closures that are fixed and must be turned before the powder can be shaken. Instructions for how to use talc products safely are listed on the package label and includes directions to keep powder away from the face to avoid inhalation, and to keep powder out of children's reach.

A helpful resource for friends and family looking for more details about talc is our Safety and Care Commitment website. If you are interested in sharing any of this information externally via social media, you can use the following link: http://www.safetyandcarecommitment.com/ingredient-info/other/talc or shortened link: http://goo.gl/mTkD6l. Please be sure to include #mycompany when you post.

From: Murphy, Catherine [CPCUS]
To: GPGGLOBALBABY
Sent: 2/24/2016 2:10:14 AM
Subject: URGENT: Talc Update

Dear Baby Professional Marketers,

Wanted to make you aware of a recent ruling around Talc in the US, our Global statement of assurance and the facts behind our products, and guidance on external communications. Please see below for the details

Thanks and please raise any questions through myself or your local management

Catherine

From: Bass, Debra [CPCUS]
Sent: Tuesday, February 23, 2016 6:13 PM
To: Allen, Marni [CONGB]; Bass, Debra [CPCUS]; Bigelow, Coleman [CPCUS]; Brissac, Daniella [CONBR]; Glasgow, Tara [CPCUS]; Higgins, Paul [JJCUS]; Khanna, Deeptha [JJISG]; Lumba, Ena [CPCUS]; Marsh, Glenda [CPCUS]; McQuade, Meg [JJCUS]; Murphy, Catherine [CPCUS]; Pizzelanti, Alice [JJCUS]; Plank, Steven [CPCUS]; Ruiz, Carla [JJCUS]; Santamaria, Luca [CPCUS]; Tull Bucaro, Wendy [JJCUS]; Urista Cardenas, Maria [CPCUS]; Weinstein, Steven [CPCUS]; Mays, David [CPCUS]; Harshavardhan, GK [JJISG]
Cc: Goodrich, Carol [JJCUS]; Dolginoff, Lori [CPCUS]
Subject: URGENT: Talc Update

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From: Mays, David [CPCUS]
To: Le, Chau Giang [JJISG]; Battistella, Sabrina B. [CONBR]; Pelino, Jose E. [CONBR]; Kwon, Robert [JJISG]; Telofski, Lorena [CPCUS]
Sent: 2/24/2016 1:17:03 PM
Subject: FW: Advisors
Attachments: 03_Talc Presentation FINAL 020514.pptx

FYI … Might be helpful.

DAVID A. MAYS, PHARMD, MBA
Senior Director, Global Scientific Engagement
Fellow, Johnson & Johnson Consumer

Johnson & Johnson Consumer Inc.

199 Grandview Rd; SC140
Skillman, NJ 08558-9418
tel 908-874-2806
mb 908-764-5100
dmays@its.jnj.com

Building and Strengthening the Foundation for a Solid Scientific Future

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From: DAVID MAYS <DMays@its.jnj.com>
Date: Wednesday, February 24, 2016 at 8:07 AM
To: "Goodrich, Carol [JJCUS]" <CGood2@its.jnj.com>, Peggy Ballman <PBallman@its.jnj.com>, LORI DOLGINOFF <LDolgino@its.jnj.com>
Cc: DEBRA BASS <DBass3@its.jnj.com>, TARA GLASGOW <TGlasgow@its.jnj.com>, DAVID MAYS <DMays@its.jnj.com>
Subject: Advisors

Carol/Peggy/Lori

I know that everyone is swamped but I wanted to pass on/remind everyone of the media training we did in 2014 with some key pediatric influencers in the US and Canada. In the slide below are the list of advisors who spent the day going through specific issues and potential conversations that may be part of their normal dialog with Patients and Consumers.

I've also attached the content that Lorena presented on Talc as a backgrounded. While there may be a few updates, the content is relevant to support the safety.

I provide this as a reminder only.

David.

Advisors

Bruce J. Brovender, MD
Global Pediatrics
Scarsdale, NY

Luisa Ciofani, RN, MSc(A), IBCLC, PNC-C
McGill University Health Centre
Montreal, Quebec

Kirk Gautier, PA-C
Dermatology Associates of Tyler
Tyler, TX

Steven B. Hoath, MD
Cincinnati Children's Hospital Medical Center
Cincinnati, OH

Paul S. Horowitz, MD, FAAI
Discovery Pediatrics
Valencia, CA

Carolyn H. Lund, RN, MS, F
Children's Hospital & Resear
Center Oakland
San Francisco, CA

Renee P. McLeod, PhD, APRN-BC, CPNP, FAA
Brandman University
San Diego, CA

Jill A. Morgan, PharmD, BC
University of Maryland Schoo
Baltimore, MD

Jo
DIVISION C

DAVID A. MAYS, PHARMD, MBA
Senior Director, Global Scientific Engagement
Fellow, Johnson & Johnson Consumer

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tel 908-874-2806
mb 908-764-5100
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Building and Strengthening the Foundation for a Solid Scientific Future

File Provided Natively

From: Murphy, Catherine [CPCUS]
To: GPGGLOBALORALCARE; GPGGLOBALBEAUTY; GPGGLOBALOTC
Sent: 2/24/2016 2:27:43 PM
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From: Mays, David [CPCUS]
To: Telofski, Lorena [CPCUS]
Sent: 3/29/2016 12:01:16 PM
Subject: FW: Toolkit
Attachments: TalcFinalToolkitMarch21.16.docx

D A V I D A. M A Y S, P H A R M D, M B A
Senior Director, Global Scientific Engagement
Fellow, Johnson & Johnson Consumer

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Building and Strengthening the Foundation for a Solid Scientific Future

On 3/29/16, 7:17 AM, "Goodrich, Carol [JJCUS]" wrote:

>Here you go David. Let me know if you have questions.
>
>Carol
>
>-----Original Message-----
>From: Mays, David [CPCUS]
>Sent: Tuesday, March 29, 2016 7:08 AM
>To: Goodrich, Carol [JJCUS]; Whelan, Kevin [CPCUS]
>Cc: Glasgow, Tara [CPCUS]
>Subject: Toolkit
>
>Carol and Kevin.
>
>I may have missed the email with details or the call but please share he
>details on the toolkit.
>
>I would like to remain consistent with the EMEA visits planned.
>
>Thank you.
>David.
>
>Sent from my iPhone

Withheld for Privilege

From: Nicholson, Susan [CPCUS]
Sent: Wednesday, August 17, 2016 1:44 PM
To: Whelan, Kevin [CPCUS]; Allen, Marni [CONGB]; Almeida, Caroline [CONGB]; Bryant, LaMont [MCCUS]; Buffat, Maxime [JJSBF]; Ekuta, Jethro [CPCUS]; Glasgow, Tara [CPCUS]; Gohlar, Meena [CONGB]; Goodrich, Carol [JJCUS]; Griffiths, Mark [CONGB]; Hicks, Don [CPCUS]; Le, Chau Giang [JJISG]; MATTOS, Adriana Paes Leme [CONBR]; Maranes, Gustavo [JPPBE]; Mays, David [CPCUS]; Raven, Stephen [CONCH]; Reynertson, Kurt [CPCUS]; Son, Rosa [JJCUS]; Szczepaniak, Lynne [CPCUS]; Telofski, Lorena [CPCUS]
Cc: Bangale, Prasanna [CONIN]; Barra, Cristina [CPCUS]; Fazio, Lil [CPCUS]; Jessurun, Christina [CPCUS]; Jousselin, Magali [JJCUS]; Li, Hua [CONCN]; Murphy, Glen [CONCA]; Roberts, Andy [CONAE]; Shah, Rita [MCCUS]; Tull Bucaro, Wendy [JJCUS]; Van Passel, David [JPPBE]
Subject: RE: Global Baby Issue Team - TALC

Gonzalez-prepub.pdf

As mentioned, the latest prospective epidemiology study.

S

Susan C. Nicholson, MD, FIDSA
Vice President Safety Surveillance and Risk Management
Consumer Products
908-745-9199 cell

-----Original Appointment-----
From: Whelan, Kevin [CPCUS]
Sent: Monday, March 14, 2016 2:55 PM
To: Whelan, Kevin [CPCUS]; Allen, Marni [CONGB]; Almeida, Caroline [CONGB]; Bryant, LaMont [MCCUS]; Buffat, Maxime [JJSBF]; Ekuta, Jethro [CPCUS]; Glasgow, Tara [CPCUS]; Gohlar, Meena [CONGB]; Goodrich, Carol [JJCUS]; Griffiths, Mark [CONGB]; Hicks, Don [CPCUS]; Le, Chau Giang [JJISG]; MATTOS, Adriana Paes Leme [CONBR]; Maranes, Gustavo [JPPBE]; Mays, David [CPCUS]; Nicholson, Susan [CPCUS]; Raven, Stephen [CONCH]; Reynertson, Kurt [CPCUS]; Son, Rosa [CONUS]; Szczepaniak, Lynne [CPCUS]; Telofski, Lorena [CPCUS]
Cc: Bangale, Prasanna [CONIN]; Barra, Cristina [CPCUS]; Fazio, Lil [CPCUS]; Jessurun, Christina [CPCUS]; Jousselin, Magali [JACFR]; Li, Hua [CONCN]; Murphy, Glen [CONCA]; Roberts, Andy [CONAE]; Shah, Rita [MCCUS]; Tull Bucaro, Wendy [JJCUS]; Van Passel, David [JPPBE]
Subject: Global Baby Issue Team - TALC
When: Tuesday, August 16, 2016 7:00 AM-7:30 AM America/New_York.
Where: telecon - CODE: Redacted: Personal Information

All,

Adjusting this standing meeting as per your recommendations. Thank you all for supporting our continued touch points. Best, Kevin

All,

It has come up in many conversations that we need a global-core-issue-team to ensure we are working, supporting and communicating in a coordinated manner on talc. Therefore, I am putting this weekly meeting on your calendar.

If key-colleagues are missing, please forward. However, in order to keep this team effective, let us be strategic in representation.

In order to facilitate updates, we are developing a global tracker in order to manage all of the global activities. I expect to send that document to you all by mid-week.

The ASK: Please make sure you have a team-representative at each meeting. This way we ensure regional representation and effectiveness.

Please do not hesitate with any questions. Thanks in advance for supporting this critically important team.

Best,

Kevin

